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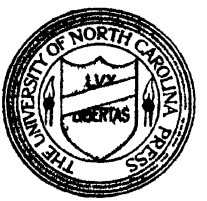

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DEDICATION OF THE WILSON ZOOLOGICAL LABORATORY

The Wilson Zoological Laboratory of the University of North Carolina was formally dedicated on Thursday, November 20, 1941, at Chapel Hill. The dedication exercises, over which Dean R. B. House presided, were arranged by a committee consisting of Dr. I. C. Kitchin, Dr. W. L. Engels, and Mr. R.M. Grumman. The Laboratory Building was open for public inspection and the portrait of Dr. Wilson (see page 8) was on exhibit in the Seminar Room.

Below are given, wholly or in part, the addresses that were delivered at the dedication exercises.

ORIGIN AND DEVELOPMENT OF THE DEPARTMENT OF ZOOLOGY

By R. E. Coker

Kenan Professor of Zoology, University of North Carolina

It is a coincidence, but a happy one, that this home for zoology should be dedicated to service and to memory on the semicentennial of the founding of a Department of Biology in the University of North Carolina, the semicentennial of the first zoological research of the modern sort in this University and the semicentennial of Dr. Wilson's arrival in Chapel Hill. We shall soon see the fitness of the designation of this year, 1941, as our semicentennial for biology in the modern sense.

More than a hundred years ago we had a Professor of Modern Languages who accomplished such notable descriptive work in the field of zoology as will keep his name and that of North Carolina in the zoological literature for years to come. The reference, of course, is to Nicholas Hentz,[1] Professor of Modern Languages from 1826 to 1833, who published not only a French reader for school and college students but also a great number of exquisitely illustrated papers on spiders, adding substantially to the knowledge of that group in America.

[1] **Cobb, Collier.** *Nicholas Marcellus Hentz*, Journal of the Elisha Mitchell Scientific Society, 41, No. 1, January 1932, pp. 47–51.

(Hentz, Nicholas Marcellus). *The Spiders of the United States*, A Collection of the Arachnological Writings of Nicholas Marcellus Hentz, M.D., xiii plus 171 pp., 30 Pls., Cloth. Boston Society of Natural History, 1875. Unfortunately Hentz seems to have regarded the name of the state, North Carolina, Alabama, Massachusetts, etc., as sufficient indication of locality of collection.

Somewhat later, the biological sciences attained a sort of back-door recognition to the extent that, in the words of the catalogue, "the sciences of Botany and Zoology receive attention," and this in a Department of Chemistry, Mineralogy and Geology. The words quoted are found without change in an even dozen catalogues from 1846–47 to 1857–58,[2] but they are followed by the express statement that these sciences receive attention only in a subordinate way. Some biology was, then, taught nearly 100 years ago by the famous Elisha Mitchell, for whom there are named a mountain, a scientific society, and a scientific journal. In the general library we have his printed outline of a course entitled: "Natural History" (27 pp.).

When the University, which had been forced to close in 1868, reopened in 1875, Biology had a somewhat better foothold as part of a School of Natural History in a College of Agriculture (not in the College of Natural Sciences). Professor John Kimberly must have been a man of considerable intellectual capacity and versatility, for he headed and in fact virtually constituted a College comprising three separate schools (but not very separate, of course) - Natural History, Chemistry, and Military Tactics.

Professor Kimberly had attended the Lawrence Scientific School of Harvard, 1853-54, and Battle[3] records that "Agassiz stated that the notes of his lectures written by him were the best ever submitted by any student since his connection with the School" (Vol. 1, p. 660). Our University had given him in 1846 what was apparently an honorary A. M. while he was a teacher in Hertford County. After first joining the faculty in 1856, he spent a year in a laboratory at the University of Berlin. He taught in the University before and during the Civil War and afterward engaged in farming in Buncombe County. We take it that Professor Kimberly was primarily a chemist and agriculturist because his chair before the war was that of Chemistry applied to Agriculture and the Arts, and Dr. Battle says that "he was a man of distinguished manners and was accomplished in the Department of Chemistry applied to the Arts" (Vol. 1, p. 785).[3]

The full extent of the knowledge possessed by "Old Kim," as he was called, we do not know, but what he taught is described in the catalogue as follows:

School of Natural History

"The instruction given in this school embraces the following subjects: Vegetable Physiology, Botany, Practical Agriculture, Meteorology, Entomology, Zoology, Horticulture, Stock and Dairy Farming, Human Physiology, History, Feeding and Care of Domestic Animals, Physical Geography."

[2] "In Natural History, the Sciences of Botany and Zoology receive attention so far, only, as is necessary to a knowledge of their methods, the classification, and the means employed for distinguishing different plants and animals from each other. A much larger portion of time is devoted to Mineralogy, and pains taken to render the Student familiar with the more common and more useful minerals." *The University of North Carolina Catalogue*, 1846–47 to 1857–58.

[3] **Battle, Kemp P.** *History of the University of North Carolina*, Vol. I, 880 pp., 1907, Vol. II, 875 pp., 1912.

Did he also teach Military Tactics? He was concerned in bringing about such an addition to the curriculum.

We may smile when we think of Professor Kimberly and his five colleagues, constituting with one adjunct Professor the entire faculty and each heading a College sub-divided into Schools. We need not forget, however, that these men may have smiled too; but theirs was a smile of hope, faith, and grim resolution. Beset with every difficulty, burdened with poverty and beclouded by political confusion, they were yet seeing great visions and conceiving future magnitudes while they were undertaking with the crudest of hand trowels to lay the foundation of a reconstructed edifice whose completion they would never see. If we take any pride in our "Greater University," with its aggregate faculty of over 600 Professors and instructors, we cannot fail to acknowledge our debt to those seven visionaries—or should we not say prophets?—who were outlining extensive but rough and provisional plans which have since been modified and extended and which we hope will always be subject to change and development.

It is indeed only a year or two later that we find a suggestion of a division of labor, although a faint one at first. Professor Kimberly's second period of connection with the University, following its reopening, lasted only about one year. William H. Smith, Ph.D., of the University of Michigan came next, but, for some reason, he remained less than a year. During his brief stay he published a pamphlet[4] giving directions for collecting and preserving animals, fossils, minerals, etc. In the third year of the reopened University, Professor Frederic William Simonds occupies the chair, or davenport, of Natural History, to teach only Geology, Zoology, and Botany, although Physiology (as well as Botany) in the new Medical School was added to his duties the following year. Professor Simonds came with the degree of M.S. from Cornell but soon obtained the degree of Ph.D. from Syracuse University (1879). Ill health required his withdrawal in 1881 from Chapel Hill and from all University work for a number of years; but he recovered and subsequently attained high rank as a geologist and geographer, publishing many books and scientific papers. He died last spring in his eighty-eighth year, having retired only a few years ago, as Professor of Geography in the University of Texas, which he had served for 52 years. Dr. Battle quotes a farmer as saying, after Professor Simonds had addressed the State Fair: "The best thing I saw at the Fair was that Chapel Hill Professor at

[4] Professor Simonds first appears in the catalogue for 1877-78. William H. Smith, Ph.D. from the University of Michigan, was listed in the catalogue for 1876-77 but with the notation "resigned." While here he published a pamphlet which contained "minute directions, such as had never been given before in this State, for skinning and preserving the skins, feathers and eggs of birds and mammals, for the preservation of reptiles, fish, insects, plants, crabs, lobsters, starfish and sea urchins, corals and sponges. Instructions were also given in regard to specimens of minerals, rocks and fossils, soils and well borings. If the directions given by Professor Smith had been more generally followed throughout the State the University Museum would have been greatly increased in value, and a practical acquaintance with it would have enlightened our people. For personal reasons Professor Smith resigned in the spring of 1877." (Battle, Vol. II, p. 123.) The pamphlet mentioned and others written by Mitchell and Simonds and employed in their teachings are in the general library.

the blackboard, drawing the figures in his lecture with both hands." Indeed there are several newspaper accounts of his artistry and his lucidity in the teaching of Zoology. The following printed outlines of some of his courses are in the general library: "Syllabus of a course of 25 Lectures on Physical Geography and Geology Delivered at the University of North Carolina, Autumn Session, 1878" (2 pp.); and "Outline of Lectures on General Geology," Raleigh: Edwards and Broughton, 1880 (10 pp.).

The place of Professor Simonds was taken in 1881 by Joseph Austin Holmes (B. Agr. of Cornell University)[5] of beloved memory. I speak with due deliberation in saying that Holmes was one of the really great men this country has produced. What he contributed to the development and conservation of natural resources, living and non-living, to the stimulation of constructive research in a dozen fields and to the preservation of human life can hardly be over-appraised. Holmes was not, however, a biologist primarily, although he taught Botany, along with Geology and Mineralogy for ten years, Horticulture and Agricultural Botany for a time, and Zoology and Medical Physiology for two periods of four and three years, respectively, ('81–85 and '88–'91).

Only four years after Professor Holmes' arrival, George F. Atkinson, Ph.B. of Cornell, was added to the Department as an Assistant Professor. Duties were now divided: it appears from the catalogue that Holmes retained Geology and Botany and that Atkinson, who was later to become a distinguished botanist and Head of the Department of Botany at Cornell University, taught in this institution only the zoological sciences:[6] including entomology, physiology, and the anatomy, physiology, feeding and breeding of domestic animals, in the Department of Geology and Natural History and the College of Agriculture. He surrendered this light task to join the faculty of the University of South Carolina in 1888.

Zoology had, then, been taught here by Mitchell, Kimberly, Smith, Simonds, Holmes, and Atkinson, all men of great merit, most of whom won high distinction in their respective fields; but the first authentic zoologist came in 1891, ten years after Holmes. Fifty years ago Henry Van Peters Wilson, after receiving the degree of Ph.D. from the Johns Hopkins University and engaging for three additional years in research, first as Bruce Fellow of Johns Hopkins and then as Scientific Assistant in the United States Fish Commission, came to Chapel Hill to head and at first to constitute a newly created Department of Biology. He brought a combination of qualities and experiences we had not had here in Biology before: the best training then obtainable anywhere in the United States, fortified by years of successful activity in independent research, an unquenchable personal zeal for the advancement of science through research,

[5] Professor Holmes' degree has been variously stated as "B.Agr." and "B.S." The Registrar of Cornell writes that the degree he received was B.Agr., as it is given in the University catalogues, but not in several biographic sketches.

[6] Atkinson was Assistant Professor from 1884 to 1887 and Associate Professor in 1887. Both Holmes and Atkinson were also Professors in the College of Agriculture and the Mechanic Arts, 1885–88.

an untiring energy and a devotion to the severest standards of integrity in teaching and research, in scholarship, and in life.

Thus the first Department of Biology in the history of the University was inaugurated just fifty years ago this autumn with the first teaching of zoology by an accomplished and confirmed zoologist. Research was immediately a part of the order of the day and it has so continued without a break. What that beginning of a one-man Department has meant to the Departments of Zoology and Botany of this day with a dozen teachers and investigators, it would be superfluous to emphasize. What also Wilson, Venable, and some of their colleagues of the nineties have meant to the scholarly integrity of the University as a whole must surely be highly appraised by anyone who has thoughtfully observed or studied the history of the University during the past half century.

If we look only at that small number of young men who served as assistants in the first ten years of the new Department, and there were only eleven of them, we find such men of later distinction as: Charles Roberson (1872–1931), a successful and notable physician of Greensboro; George H. Kirby (1875–1935), long Professor of Psychiatry in Cornell University Medical School, Director of the New York Psychiatric Institute, and Consultant to the New York State Department of Public Health and the United States Public Health Service; Edward Jenner Wood (1878–1928), distinguished investigator of tropical and sub-tropical diseases, author of important medical texts; William deBerniere MacNider, to become Dean of our Medical School, eminent in research and a member of the National Academy of Sciences; T. Gilbert Pearson, author of several books and lately president of the National Association of Audubon Societies, and the greatly loved Dr. Clarence A. Shore, for whom the State Laboratory of Public Health was recently named almost by acclamation. Shore by the way was the first to bear the title of "Instructor in Biology" in the Department of Biology (1901–02), although J. V. Lewis and Victor S. Bryant had borne the same title with Holmes in the Department of Natural History, 1899–1900. If we could go on just a little into the next decade we should name Dean Ivey F. Lewis, Dr. J. K. Hall, Dr. Fred M. Hanes, and others, but we must stop somewhere.

But, to go back a little, it was Wilson and Holmes together who, with the officers of the U. S. Fish Commission in Washington and members of the Congress, brought about the establishment at Beaufort, N. C., in 1901–02 of the first permanent marine biological station on the South Atlantic coast. Seaside study and research, principally at Beaufort, had already been made a part of the general program of the Department of Biology. During all this time (from 1883, at least, to 1908) the Department of Biology was occupying the fourth floor of the New East Building, the central and larger part of which had previously been the Library of the Philanthropic Society.[7]

[7] The central part of this floor, which included the main laboratory of biology, comprised: a big central space having tables and sinks; three alcoves on each side with three or four tables in each alcove and with the old built-in bookshelves filled with specimens dry or in

A significant development of the Department came in its eleventh year (1902–1903) when Dr. W. C. Coker joined the staff as Associate Professor of Botany. Just a few years later, in 1908–1909, he became Head of a Department of Botany, Professor Wilson retaining Zoology, as the original Department of Biology was divided into two, both of which were established in that year in the new Davie Hall. Davie, with two stories, an unfinished basement and a partially finished attic, was an excellent building for the time, and Zoology continued to occupy part of it until 1940.

We must pass rapidly over that period of thirty-two years in Davie. Sound teaching and research in Zoology were uninterrupted while the University grew greatly in size and complexity and the number of students increased more than five-fold, from 800 for 1908–09 to 4,163 for 1939–40. The increasing demands upon the two biology departments in Davie could hardly be met. A second professor, the present speaker, was added to Zoology in 1922. A three-story Botany wing was attached to the building in 1924–25. Dr. C. D. Beers joined the staff of Zoology in 1927, as Assistant Professor, later to become Associate Professor and then Professor. We are brought to the year 1935, when Dr. Wilson at the age of 72 years found it desirable to relinquish administrative responsibilities as head of the department while continuing to teach and to engage in research until a few days before his death in January, 1939. He had given up undergraduate teaching in 1937–38.

Dr. D. P. Costello was added to our staff in 1935, Dr. W. L. Engels in 1937, and Drs. I. C. Kitchin and R. D. Boche in 1939. Meantime the two Departments had become impossibly crowded in Davie. Zoology had already overflowed into the basement of the Pharmacy Building without being able to accommodate by any means all the students desiring or needing training in our field. Necessarily we began to work on plans for a building and facilities that would permit this Department and that of Botany to render their appropriate services. What we all wanted at the outset was a new building for the two departments, a biology building; but it was soon decided definitely, if regretfully, that a new classroom building was beyond the possibilities of the time. The best that could be proposed was that Zoology should be moved into Caldwell, the old medical building, prospectively to be renovated, fire-proofed and enlarged as necessary to serve the purpose of a departent requiring laboratories for a substantial number of students and protection against fire for its invaluable library, scientific equipment, and biological materials. Our removal would, of course, permit expansion for the Department of Botany. As unsatisfactory as this arrangement promised to be at the best, it was nevertheless the only discernible solution for a condition of crowding and inadequacy that cried too loudly for relief. Plans were laid for the expected change and appropriations secured by the Administration, in part from the State and in part from the Public Works Administration.

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bottles; an instructor's laboratory, a store room, a tank room, and an entrance hall with exhibition cases. In the small wings, which were at a somewhat lower level, there was at the east end a lecture room and at the west end an advanced laboratory and Dr. Wilson's private laboratory.

After the appropriations became available there followed about a year during which architects and Buildings and Grounds Committee, Administration, and Department, labored indefatigably to work out a plan for renovation and enlargement of Caldwell to meet in some fashion the teaching need of the Department without defacement of the campus. Repeated attempts to carry out the general plan failed to win approval from any source. In the end it seemed inevitable, either that provision of the required space for Zoology and Botany must be abandoned, or else that there must be thought of a new building for Zoology in a new place. Then, under the leadership of President Graham, with the unanimous vote of the Building Committee, the approval of the Governor's Council of State, and, finally, the authorization of the Public Works Administration, this new building came to be envisioned on what we think is the most fortunate site on the campus. We are here, however, not because of the beauty of the location, but rather because this was the most readily available site and because the location fitted admirably into a zoning plan that might ultimately put all of the departments of the natural sciences within a continuous area extending from Phillips, with Mathematics and Physics on the North, to the Medical and Public Health centers on the South.

The delay may have been wearing, more particularly on the Buildings and Grounds Committee, which had the burden of long meetings and painstaking examinations of a dozen different plans. In the end the delay was perhaps one of our greatest pieces of good fortune. The realization of the new building for Zoology came almost at the conclusion of the general building program. Contractors and furnishers of materials had begun to see the end and to develop eagerness for new work. Prices had so fallen that we actually obtained a better-finished building than we had ever dared to hope for.

Finally we moved into the new building just as work was beginning for the spring quarter in 1940. We had the space but we could not immediately use it because our scientific equipment was only just what we had had before the move. Throughout a large part of this building there were tables with no microscopes and, in consequence, seats and rooms with no students. The Department in that one quarter was like a starving man who suddenly found himself provided with an abundance of food in a locked room and no key. Then within a few months came another stroke of good fortune when, by the aid of the General Education Board, the Administration and the Trustees of the University, we were provided with microscopes and other equipment that more than doubled our capacity to teach in so far as it had been limited by material facilities. The equipment was none too much and came none too soon, for the enrollment of students availing themselves of building and equipment increased from about 600 in 1939–40 to more than 1,000 in the next year and the first in this building (counting each registration as one). We need not attach importance to numbers but nevertheless we do not like to have to say to students, as we did less than a year and a half ago: "There is no place for you in our classes."

We have looked back over a half-century and more to scan very hurriedly the path by which we have arrived at this point. There has been growth in numbers of students exposed to the presumed benefits of instruction in respect

to animal and human biology, as the following approximate figures of departmental enrollment at ten-year intervals show. (The Department of Zoology proper dates from 1908.)

1910–11	134 plus 30 medical students
1920–21	125
1930–31	262
1940–41	1049

But in educational work there are real dangers in numbers.

We have looked back, but it is even more important now for us to look seriously along the path that lies ahead. The retrospect should only stimulate us to face the prospect. Accepting gratefully our physical gifts, our increased enrollment and correspondingly greater opportunity to render educational service, and our scientific and spiritual heritage, we may well think what is to be done now and in the future. How sound will be our teaching and scholarship and how significant our research during the half century ahead? That is our present and future responsibility.

THE WILSON MEMORIAL FUND

By Kemp D. Battle

Chairman of the Wilson Memorial Fund Committee

Shortly after Dr. Wilson's death, a number of people expressed the wish that suitable means might be found to perpetuate his memory by a memorial in the University. A committee was organized consisting of President F. P. Graham, Dean R. B. House, Dr. W. C. Coker, Dr. R. E. Coker, Dean Ivey F. Lewis of the University of Virginia, and myself. Dr. R. E. Coker served as treasurer and I acted as chairman. The committee decided first to give an opportunity to the friends of Dr. Wilson to participate in a memorial fund and then to determine the nature of the memorial in the light of the amount available. No systematic canvass of the alumni was made but selected individuals in the classes which Dr. Wilson taught were invited to participate. A total of approximately $4600.00 has been contributed by 155 persons, nearly two-thirds of the amount being given by Dr. H. V. P. Wilson, Jr.

The committee decided upon a portrait of Dr. Wilson, which was to be placed in the Wilson Zoological Laboratory, and an endowed scholarship in marine biology. The members of Dr. Wilson's family acquiesced with some reluctance in the decision regarding the portrait, feeling that it would be more in keeping with his wishes to devote the entire sum to a scholarship. The committee concluded however that the portrait would be in accord with the desires of most of the contributors and would have a distinct inspirational value in the lives of students in biology, and that the reduction in the amount of the scholarship yield because of the portrait would not be significant. Miss Alice Kent Stoddard of Philadelphia was commissioned to paint the portrait, and in view of the un-

stinted praise which the likeness of Dr. Wilson has received, the committee feels that its choice of an artist was a fortunate one. The remaining portion of the fund was turned over to the University as a permanent endowment for an annual scholarship in the field of marine biology. At present rates of return it will yield $100 per year with some margin for contingencies.

It is my privilege on this occasion, President Graham, to present through you to the University which Dr. Wilson loved, to the State in which he spent nearly his entire working life, and to the World of Science whose borders he extended, the portrait and the memorial fund.

H. V. WILSON AS A TEACHER

BY IVEY F. LEWIS

Miller Professor of Biology, University of Virginia

At least two of us in this audience are old enough to remember the days when Chapel Hill and the University were, like the rest of North Carolina, still reaping the fruits of the Civil War. In the nineties, whose alleged gaiety lay in the hearts of an unconquerable people, the University was struggling to survive attacks from without and poverty from within. But there was more than that to the picture. The ideals of scholarship and service in a framework of democracy had come down from an older University. They were being ingrained so deeply in the life of the institution that they have been able to survive the severest of all tests, the test of prosperity, and are as characteristic now as they were then of the Carolina spirit.

It would be a pleasure to call the roll of the old faculty who, even before the phrase was invented, were able to carry on with unimaginably small resources and hold the torch high with the dauntless courage of those "few stout and earnest persons," the record of whose actions we call history. In addition to men of talent who served the University well, such as Cobb and Gore, Whitehead and Holmes, two must be especially mentioned. F. P. Venable caught the research spirit in the universities of Germany and brought it to this quiet spot. H. V. Wilson came from the first American university in the modern sense of the word. Between them they introduced modern science to the University.

The man whose memory we honor today in this magnificent laboratory of zoology was teaching in 1899 on the fourth floor of the New East building. He was at that time something of a terror to his students. His exacting standards were something new to us. If we did not get something just right it was a small matter to us until he saw what we had done badly. Then with quick incisive words he would set us straight. There was never any question as to who was boss, except in one instance. A 200-pound student in histology came into his office with a complaint that seemed to him to have no merit. He made his decision, but the student remained to argue. As voices rose, we slaves of the microscope in an adjoining room were startled to hear in clarion tones, "Get out now or I will throw you out of the window." The mental image of the 100-

pound gamecock seriously proposing to throw the 200-pounder bodily out of a window remains to this day a source of chuckles.

Dr. Wilson, however, loved an argument. I see now that these were often used to sharpen intellects, one of his devices for encouraging precise thinking and accurate statement. He was careful in his own thinking and wanted us to be likewise. He would search long for the exact word to express an idea with precision and clarity.

One of his qualities that made him a great teacher was his keen appraisal of the men. When I came into his laboratory his assistants were Dorman Thompson, Clarence Shore, and James K. Hall. Those who knew these men would, I think, be unanimous in agreeing that they were men of unusual quality. In handling laboratory classes the assistant is almost as important as the professor, and Dr. Wilson was a discriminating judge of men.

He was sharp and quick in his judgments. Once when I had struggled for two hours to produce a good drawing he glanced at it and turning to Clarence Shore, remarked, "I have never been able to understand why the students in the Goldsboro school can make so much neater and more accurate drawings than University students who are older and presumably better trained." The mystery of it did not seem to us so strange when we learned that the teacher in the Goldsboro school was Robert E. Coker.

The nickname "Froggy" was firmly established in the current *mores* when I first knew Dr. Wilson. We were afraid he would resent it, but there was nothing small about him except his size. There was a decidedly jolly side to the strict scholar, though he did mildly resent the appellation of "Tadpole" bestowed on his small son.

It was at the Fisheries Laboratory in Beaufort that Froggy was seen at his best. There is something about salt water that brings out the free play and full expression of personality. Inhibitions are left behind in the presence of our primeval mother, the ocean. His discussions under the relaxing sun and wind and water of Piver's Island were an important factor in his success as a teacher of older students. It was a privilege to hear him debate a philosophical point with his old teacher, W. K. Brooks, and his occasional explosive and impassioned arguments with Caswell Grave were something to which we minor satellites looked forward with eager interest, even though we feared lest mayhem inject its ugly head. It never did, though, and the protagonists would invariably simmer down to the friendly relations of two people who genuinely liked each other.

The Beaufort Laboratory was his child, his joy and pet. Teaming up with Joseph A. Holmes, Dr. Wilson was irresistible. Wilson would find and arrange the ammunition which Holmes would fire with an effectiveness that was astonishing. Brooks moved his Chesapeake Laboratory to Beaufort, and it was soon seen that here was an extraordinarily favorable spot for a marine laboratory. When the establishment of the Bureau of Fisheries Laboratory became a fact, Dr. Wilson's joy was unbounded. He was nurtured in the Hopkins tradition that every graduate student in zoology should have direct experience with marine

life. That work at Beaufort was actually a rich and indispensable experience is the verdict of the years. This is due in part to the wealth of material and its suitability for experimental work, but also to the intimate association with men who in the winter are too engrossed with routine duties to have time for leisurely conversation. The afternoon swims and the evenings on the porch hearing interesting and at times inspiring discussions, did much for the student at Beaufort.

It was at Beaufort that we came to know the man as something more than the teacher. At Chapel Hill we knew him in the classroom and laboratory, and we knew that he devoted much of his spare time to taxonomy of sponges, with occasional turns at the embryology of the frog and other things. At Beaufort he was more than the observer and classifier. He developed experimental methods that soon resulted in significant and important discoveries. There is no instruction for graduate students comparable with association with a productive research man whose methods you can observe and whose results become visible. It takes a very unusual combination of talents to become a world authority on the classification of so difficult a group as the sponges and at the same time an experimenter of the first order. It is no wonder that before he died Dr. Wilson became one of that lamentably small group of Southern scientists elected to the National Academy of Science.

As a teacher of undergraduates, Froggy was characterized by the strictest attention to the subject in hand. He was not a purveyor of cheerful facts. If he had been a teacher of history he would never have digressed to give a remedy for snake bites. If a geologist, he would not have defined a gold mine as a "hole in the ground owned by a fool." As a matter of fact, that definition is as meaty as any I ever heard, and I cherish the recollection of the *bon mot* when I have forgotten more rigorous and less lively statements. In general, Froggy depended not on books, but on his own lectures, delivered with speed and precision. It was a real workout to take his notes, but when you got them, you had a full, concise, clear statement of the existing knowledge on the subject. Fortunately he had learned, I suppose from or with his friend E. A. Andrews, the technique of making rapid and effective diagrams. His reconstructions in colored chalk of the stages in the embryology of the chick were marvels to behold. It was too bad that we students were pushing our pencils so far beyond the speed limit that we could spare only the most cursory glance at the blackboards loaded by the end of the lecture with the elaborate cabalistics of animal morphology.

The truth is that the text books, at least in embryology, were rather poor in those days. Whether with the appearance of better books he modified his methods I do not know.

The completeness and fine finish of his lectures once led me to an error of judgment, which I may mention in this intimate circle of friends. It occurred to me that if I knew shorthand I could take down his lectures word for word and still have time to look at his diagrams, so I took time off and learned the elements of stenography under David Graham. Full of zeal, I took down my first lecture, only to discover that in the exercise of a specialist's art I had not understood one

word of what went on. My career as a stenographer ended on the first day, but the incident will illustrate how valuable the lectures seemed to the young student.

All in all, Dr. Wilson was one of the most independent men that ever lived. He was the perfect illustration of the old saying, "the game fish swims upsteam." He would not follow the crowd, and his refusal to identify himself with any group led to delay in national recognition of his services to zoology. It did not matter to him. His beacon was an inner light within his own breast. Like Ulysses he followed "knowledge like a sinking star"—"strong in will to strive, to seek, to find, and not to yield." His independence showed itself in the outward man. A certain jauntiness of bearing was merely the outward and visible sign of an inward and spiritual quality. There was no compromise in him, no traffic in half truths, no tolerance for error. He was true to himself.

Students sense these things even when they do not put them into words. They know quality by instinct and are geniuses for distinguishing between the sham and the real. By every test Dr. Wilson rang true. The quality of the man himself was what counted most in his influence on his students and in his service to the University. His keen intellect, his rigorous self discipline, his devotion to the truth as he saw it, his complete independence—these are among the qualities of the man that earned the respect and affection of his students. Long after they have forgotten the scientific details of his teachings, they yet cherish as a living force in their own lives the example and the ideals of a great teacher.

H. V. WILSON'S SCIENTIFIC CONTRIBUTIONS

By C. D. Beers

Professor of Zoology, University of North Carolina

The first in the long series of publications that constitute Dr. Wilson's contributions to science appeared in 1886, while he was still a graduate student in the Johns Hopkins University; the last that he himself completed, on certain aspects of experimentally induced degeneration in sponges, appeared in 1938.[8] The fifty-three years that intervened between 1886 and 1939, the year of his death, were years of ceaseless and untiring investigative activity, for in this period he published a total of approximately seventy-five articles of major scientific import, a dozen or more reviews and biographical sketches, and a translation of a part of Metschnikoff's classic studies on medusan embryology. These contributions, noteworthy in themselves, seem all the more remarkable when it is recalled that they were made not by a full-time investigator in a research institution but by a university teacher and administrator whose time was already well occupied by instructional and routine duties. Indeed, they were completed only through the efficient utilization of the summer vacation periods

[8] Dr. Wilson's complete bibliography, except for two titles, may be found in the Journal of the Elisha Mitchell Scientific Society, vol. 50, pp. 411–415 (1934) and vol. 55, pp. 5–6 (1939). The missing titles are the following: "Sara Gwendolen Andrews," Science, vol. 85, p. 213 (1937), and "The Recapitulation Theory or Biogenetic Law in Embryology," American Naturalist, vol. 75, pp. 20–30 (1941), the latter being a paper edited by his colleagues from longhand notes and published posthumously.

and through the wise allocation and judicious employment of practically every working hour of the academic year.

Dr. Wilson's early investigations dealt with various aspects of normal development and adult structure in hydromedusae, corals, and sea anemones. The observations were made largely in the Bahama Islands, which he visited on two occasions, and at Beaufort, N. C., during his four years, 1885–89, as graduate student and Bruce Fellow in the Johns Hopkins University. Of these early investigations the most important was his doctoral dissertation, presented for the degree in 1888. It concerned the early embryogeny and larval development of a stony coral, *Manicina areolata*, and in its final published form was beautifully illustrated with seven lithographed plates, the original drawings for which were made by Dr. Wilson himself. He published in all eight papers on marine coelenterates.

In 1889, while employed as Scientific Assistant in the U. S. Fisheries Laboratory at Woods Hole, Mass., his interest turned to vertebrate embryology, and he completed in his two years at Woods Hole (1889–91) a very thorough and precise study of the embryology of the sea bass *Serranus atrarius*. The published results of this investigation comprise sixty-eight pages of text and twenty plates of original figures. Some of these illustrations are still to be found in current textbooks, and the study has long been regarded as a model for the guidance of beginning investigators in descriptive vertebrate embryology. While his interest in this subject persisted throughout his life, and while he read prodigiously in the literature of experimental embryology, he published only six additional papers in this field. Two of these, on the formation and closure of the blastopore in the frog egg, occasioned more than ordinary comment, in that the observations, as interpreted by Dr. Wilson, seemed to cast doubt on the validity of the Roux-Hertwig-Morgan theory of the formation of the axial embryonic body by concrescence. In order to observe the normal growth of the blastopore lips over the yolk mass in the living frog egg, Dr. Wilson used an inverted microscope which had been custom-made to his specifications. This remarkable instrument, comprising a system of lenses and prisms *beneath* the stage and a mirror on a long, flexible arm *above* the stage, has never failed to evoke the special interest and favorable comment of every microscopist who has examined it.

It is clear that when Dr. Wilson came to Chapel Hill in 1891 his interest in the Porifera, later to become the dominant one of his research career, was already fairly well established. His first paper in this field, describing some phases of development in sponges, appeared in 1891, and it was followed in the succeeding ten years by four further contributions to our knowledge of the general morphology and development of marine sponges. In 1902 the first of his major contributions to the comparative morphology and taxonomy of sponges appeared, a report on a collection taken in Puerto Rican waters. Fourteen new species and six new varieties are described, and the report, in keeping with Dr. Wilson's well-known penchant for the correct and unambiguous use of language, contains the definitions of some eighty technical terms relating to the spiculation and general morphology of sponges.

In 1904 there appeared in quarto form the most handsomely illustrated of his taxonomic works, a report on the sponges taken by the U. S. Fisheries Steamer "Albatross" off the West Coast of Mexico and Central America. Much of the work on this monograph was done in the year 1902–03 in Professor F. E. Schulze's laboratory in Berlin, after visits to the important museums of Europe to examine type specimens. One genus, twenty-four species and nine subspecies new to science are described, and twenty-six plates of photographs and lithographs accompany the 164 pages of text. To aid him in the preparation of the illustrations Dr. Wilson engaged the services of a professional artist. However, those who were well acquainted with Dr. Wilson will readily understand that his faithful adherence to precise factual detail would scarcely be compatible with the interpretive license of the artistic temperament. Hence, the combination of scientist and artist was not at all harmonious, and while the illustrations of the early part of the monograph were the result of a joint effort, those of the latter part were made by Dr. Wilson alone, the artist no doubt having preferred to seek his livelihood under less rigorous circumstances.

Leaving now for a period of approximately fifteen years (1903–18) the fields of comparative morphology and descriptive embryology, his work became largely experimental, for he began in 1904 or thereabouts a critical examination of the regenerative capacities in marine sponges. In the most striking of these experiments, the results of which first became adequately known to the world of science in 1907, pieces of the red oyster sponge, *Microciona prolifera*, were broken up into their component cells by the simple but ingenious expedient of pressing them through the meshes of fine silk bolting cloth. The procedure proves to be far less injurious to the cells than might be expected, since the elastic recoil of the skeletal elements opposes the pressure experimentally applied in such a way as to protect the cells against extremes of compression. The many types of dissociated cells when allowed to settle in clean sea water onto micro-slides form at first merely a disorganized red sediment. Soon, however, the cells begin to show ameboid movement and to fuse one with another to form small aggregates or conglomerates which in structure and behavior resemble plasmodia. Such aggregates then coalesce with one another and incorporate free cells, thus increasing in size. Finally, they attach firmly to the slide, and such attached conglomerates, when transferred to live boxes under favorable conditions, assume within a week the character of small, encrusting sponges, with pores, canals, flagellated chambers and oscula. Within a month the sponges are thicker and the characteristic skeletal elements are present; at the end of another month of growth they usually have some upright branches and are producing germ cells. Physiologically and structurally they are normal sponges.

These highly original findings naturally attracted wide attention, and the results were soon confirmed by European investigators, one of whom, Karl Müller, applied the method to freshwater sponges with parallel results. Dr. Wilson himself, in further investigations, studied the behavior of the reunition masses in other genera of marine sponges and in the hydroids *Eudendrium* and *Pennaria*, in general with positive results, though the results of his attempts to produce interspecific conglomerates in sponges were largely negative.

The fundamental discovery that the dissociated cells of sponges will recombine to form restitution masses and perfect adults introduced a wealth of inviting, though difficult, experimental problems in cell mechanics and cell physiology, for example, the roles played by the different types of cells, the degree of dedifferentiation of the cells, and their structure, behavior and interdependence during regeneration. To the resolution of many of these problems, and to further taxonomic and embryological studies, Dr. Wilson, in collaboration with his students, devoted the research efforts of the last twenty years of his life.

In 1919 he published with W. C. George a report on the sponges of Beaufort, N. C., harbor and vicinity in which thirteen new species are described. The last of his major taxonomic works appeared in 1925, a report on the silicious and horny sponges taken by the Steamer "Albatross" in Philippine waters. One new genus, thirty-eight new species and nineteen new varieties are described for the region.

At this time Dr. Wilson's renown as an authority on sponges was so wide and the number of specimens sent to him for identification each year from all parts of the world was so great that their determination, involving always the preparation and study of sections, became a physical impossibility. He was therefore compelled, however reluctantly, to discontinue, except in unusual cases, the identification of the ever increasing number of sponges that found their way each year into his laboratory. Indeed, he remarked on one occasion that he would prefer never to see a sponge again as long as he lived. However, he not only saw many more of them but continued to work with them as diligently and enthusiastically as ever, for in the years 1928–1930 he completed with J. T. Penney a series of four papers chiefly on cell behavior during the regeneration process. In 1929–30 he spent his second year in Europe, engaged at the Naples Zoological Station in a critical study of the metamorphosis of halichondrine sponge larvae, the results of which appeared in print in 1935. In all he published approximately forty papers on embryology and regeneration in lower invertebrates and fifteen on the comparative morphology and taxonomy of sponges.

While it is undoubtedly the commendable ambition of perhaps every investigator to discover and develop in his own right a new and fruitful field of scientific endeavor, it is a fact that few investigators, whether as a result of experimental misadventure or inadequate inherent preparation, experience the reward of ultimate success. It may truthfully be stated, dispassionately and in terms of simple fact, that Dr. Wilson, through a fortunate combination of sharp analytical powers, unusual investigative acuity and diligent and systematic effort, succeeded in his studies on regeneration in opening up an entirely new and highly productive field of investigation.

Concluding the exercises, President Frank P. Graham spoke briefly in appreciation of Dr. Wilson as a man, as a teacher, and as an abiding influence in the life of the University. On behalf of the Board of Trustees he accepted the memorial fund and the portrait of Dr. Wilson, and, finally, named the building The Wilson Zoological Laboratory and dedicated it to the memory of one who spent his life in the cause of science.

A STUDY OF WESTERN WATERMOLDS

By James Vernon Harvey

Plates 1–8

Between December 12, 1935, and February 4, 1941, 604 samples of water or soil or water and soil were taken in southern California for the purpose of isolating species of the Saprolegniaceae. In addition to those from southern California, 46 samples were obtained from central and northern California, eight from Oregon and Washington, fifty were secured from northern Arizona and southern Utah, in the neighborhood of Monument Valley and from the San Juan and Colorado Rivers,[1] and 38 were taken from southern Arizona. Prior to the above period, between 1931 and 1935, many samples of soil or water or both were taken from various sections of the west: Glacier National Park, Montana (50 samples), Yellowstone National Park, Wyoming (50 samples), and San Juan Island, Washington (20 samples), as well as desert sections of California. With the exception of one species,[2] no other members of the Saprolegniaceae were isolated before 1935 from the areas mentioned.

Not a single species of the Saprolegniaceae was obtained from the total of 83 samples of soils and water taken in the states of Utah and Arizona. However, *Allomyces javanicus* Kniep[3] was taken from moist sand in Monument Valley, elevation about 5000 feet, and *Allomyces cystogena* Emerson[3] was obtained from dry, cracked, surface clay from the Gila River, near Yuma, Arizona.

The absence of the true watermolds from the plateau region of Utah and Arizona might be explained by the elevation, all stations being above 5000 feet, or by the aridity of the region. Samples of wet soil and water taken from the San Juan and Colorado rivers, as low as 3300 feet, likewise produced negative results. However, these streams are laden with much fine sediment, and the water flows very swiftly. From the clearer streams of tributary canyons there were no watermolds found, although algae were plentiful. *Pythium deBaryanum*, one other species of *Pythium*, and an occasional ascomycete and zygomycete were taken from the plateau region, but were missing altogether from the river collections.

The absence of water molds from the twenty collections of soil taken at Friday Harbor, San Juan Island, Washington, at or near sea level, might be explained

[1] During the summer months of 1937 the writer was privileged to be a member of the Rainbow Bridge-Monument Valley Expedition in its fifth season of exploration in the area named. As botanist, he had ample opportunity to gather specimens of soil and water from the semi-arid region. The river samples were taken during an approximately 150-mile trip through deep canyons of the San Juan and Colorado Rivers, from near Mexican Hat, Utah, to Lee's Ferry, Arizona.

[2] This mold, obtained from Glacier National Park, was written up as a well defined new species of *Isoachlya*, but upon loss of the living culture, the description was not published.

[3] The author is indebted to Dr. Fred T. Wolf for his assistance in identifying species of *Allomyces* referred to in this paper.

by the disconnection of the island from the mainland, by the latitude, or by the fact that samples favorable to the growth of such molds were not obtained.

From first meager results, prior to June 1939, it appeared as if Saprolegniaceous species were not to be found, or at least scarcely found, at high altitudes or latitudes, even though moisture conditions were ideal. From the 50 collections in Glacier National Park, Montana, at elevations of 4800 to 5000 feet, there was isolated one watermold, which was so delicate that it did not live long after cultivation in southern California.[4] In fifty samples of earth taken in Yellowstone National Park, Wyoming, at elevations of 7000 to 7700 feet, no species of the family in question appeared. Twenty samples of humus soil taken from a forestry nursery, near Placerville, California, produced nothing in the way of watermolds. From the state fish hatchery, at Mount Shasta, California, eight vials of wet soil taken from a stream, produced no desired molds. In all of the above cases, *Pythium* species, zygomycetes, ascomycetes, fungi imperfecti, and algae were reasonably plentiful.

In southern California, prior to June, 1939, most of the isolations were made from 262 collections taken mostly below 5000 feet of elevation. The elevation of San Bernardino, at the San Bernardino Valley Junior College is about 1100 feet. Soils and water specimens from San Bernardino and vicinity were rich in Saprolegniaceous molds. Likewise, samples from all other lower altitudes produced good results. As one increased his elevation, watermold isolations apparently became fewer and fewer, with the possible exceptions of certain species, locally. At Lake Cuyamaca, in San Diego County, elevation 4600 feet, an unfruiting *Aphanomyces* was found to abound. From Big Bear Lake, in San Bernardino County, elevation 6750 feet, only one specimen of *Saprolegnia* (non-fruiting) was obtained, while at nearby Baldwin Lake no member of the family had been taken. Above 5200 feet, in the Forest Home neighborhood, in the San Bernardino Mountains, no watermolds had been isolated, although from Mill Creek canyon, about one-half mile below Forest Home, elevation about 4500 feet, species were taken. The writer isolated *Aphanomyces* (non-fruiting) several times and *Brevilegnia megasperma* (fruiting) once from the Idyllwild district, elevation 4430 feet, on the flanks of Mount San Jacinto, in Riverside County. Samples of soil and water from other places in the Idyllwild area, at elevations up to 6000 feet, produced no Saprolegniales.

During the twenty-month period, ending February 4, 1941, the author made 342 collections of water or soil samples from many new localities in southern California, as well as from previously surveyed areas, thus more than doubling the total number of samples collected prior to that period, bringing the count to 604, as given in the first paragraph of this report.

In this renewed study, in a partial attempt to answer a question concerning the geographical distribution of Saprolegniaceous species in southern California and adjacent areas, the writer found some species of the family to be quite cosmopolitan. In other cases, species previously observed to be locally plentiful were found to be apparently endemic to the localized area, while still other

[4] See the second footnote on the first page of this report.

members of the family displayed an inverse ratio in numbers, when the greater area was considered. Such an outcome was somewhat expected.

It might be recalled that in southern California, there are localized "island areas," with which are associated endemic species of phanaërogamic plants, and there are interesting overlappings of localized species. The author has tried to correlate the distribution of the Saprolegniales with such a distribution scheme, but in his effort he has not been wholly successful. Many more collections of material need to be made.

With the exception of *Aphanomyces balboensis* n. sp. and a fruiting strain of *Dictyuchus*, specimens from which descriptions are made have been grown continuously in pure culture from the time of their first isolation, except for occasional periods of storage in vials. Most species may be so stored for at least six months. Storage problems are now being investigated by the writer.

SAPROLEGNIA

In all species of *Saprolegnia* isolated since the beginning of this study, there have been observable in every water culture two distinct types of mycelium, produced according to age. After a few hours, usually about twelve, a very delicate, almost transparent mycelium may be seen growing from the hempseed substratum. Its hyphae bear small sporangia but no sexual bodies. This delicate mass of threads is short-lived, for not more than two or three days, and is soon overgrown by the more pronounced, stouter hyphae of the secondary (or permanent) mycelium. Such a growth-characteristic of the genus *Saprolegnia* has been overlooked by previous workers or considered insignificant by them or possibly the development of such a delicate, transparent, primary mycelium might be considered as characteristic of the genus only in the particular geographical areas under consideration in this report. The author himself, in his earlier studies, does not recall having seen such a primary growth, but the lack of observation might easily be explained as merely an oversight. The habit details of these fine hyphae appear to vary somewhat with the species, and hence those data should be included in the descriptive text of a species. Numerous hyphal transfer cultures, as well as single spore cultures, have been made from these early one-day-old growths and the desired colonies ultimately procured therefrom. The secondary growth usually appears on the second day, at first generally as stout, radiating threads, which later develop into the mycelium of the mature plant. In giving descriptions of *Saprolegnia* species, the writer will refer to the sporangia formed from the early, more delicate hyphae as "primary," while the term "secondary" will be confined to those sporangia that arise from the so-called permanent hyphae. When sporangia are transformed from gemmae, suitable terminology will be used in describing them. The differences among the three types are sometimes greatly pronounced.

Saprolegnia diclina Humphrey

With a few exceptions this species is comparable to Coker's specimen (1923). The following partial description is necessary:

Primary mycelium up to 25 mm., over-all growth, within 24 hours, transparent; hyphae very delicate, barely distinguishable to the naked eye, profusely branched. Primary sporangia plentiful after 24 hours, cylindrical to barrel-shaped, sometimes broader at the base and tapered distally into a long papilla, or at times branched, not so large as secondary sporangia, generally about 60–160μ in length, commonly with two or three rows of spores. Secondary mycelium up to nine or ten millimeters, over-all growth, within 24 hours and to 24–26 mm. within one week; prominent, white, becoming opaque or with numerous small, white, opaque patches of gemmae and oogonia scattered throughout the older portion; hyphae crooked, gradually tapering toward a hyaline tip or ending in a sporangia-like body, not freely branched. Secondary sporangia never plentiful, 400μ or more in length, generally largest at middle or below the middle, sometimes approaching spherical, often tapering distally, frequently constricted at places; hyphal proliferation commonly of the Achlyoid type, by sympodial branching, less frequently through empty sporangia. Spores at rest 10.5μ, as contrasted with 11–11.5μ for the Chapel Hill form. Gemmae not so abundant as in the Chapel Hill form, and not tending to be commonly long and pointed as in that form, although such gemmae are present, but characteristically short and stocky, spherical to pyriform, commonly in chains of 3 to 8 or more. Oogonia and oospores as described by Coker (1923).

Isolated only four times: originally, together with the female strain of *Achlya heteromorpha* n. sp., April 13, 1938, from Warm Creek sand and water, underneath the Sixth Street bridge, east of San Bernardino; subsequent collections from the same station have not yielded the mold. It was taken again from a small stream, in a sedge thicket, at Twenty-Nine Palms, on the Mojave Desert, in San Bernardino County. The third and fourth isolations were made about one mile apart, both from sand and water, July 31, 1940, from Cajon Creek at Cozy Dell and from Horsethief Canyon at Palmer's, elevations 2800 and 3000 feet, north of San Bernardino, on U. S. Highway #66.

Saprolegnia delica Coker

The California isolates of this species agree in nearly every respect with Coker's description (1923), the sole difference lying in the characteristics of the oogonia. The following brief description, including the primary growth, is given:

Primary mycelium lax and delicate, hyphae very small. Primary sporangia, formed during the first two days, not numerous, up to 96μ in length, from hyphae 9–10μ in diameter; mostly oval to obovate or elongated, occasionally spherical. . . . Oogonia larger than in the Chapel Hill form, 64.4–99μ, occasionally up to 114.4μ, seldom larger, in contrast to 40–63μ (averaging 60μ) when grown on a fly; oogonial pits larger in the California form, generally about 7.2 x 10μ, as contrasted with 3.7–8.5μ for the Chapel Hill form.

The species has been taken seventeen times in southern California: Scenic Drive Marsh (Warm Creek), several times, the first isolation being made December 17, 1935, as well as from Devil Canyon, and from a marshy area on South Waterman Avenue, near Mill Street, all near San Bernardino; twice from Palomar Mountain, San Diego County, in sand-water samples from a meadow stream, two and five miles below the new observatory; at elevations of

4840 and 4105 feet, respectively, in mud and water from Howards' Gulch, near Canby, California; in water and clayey mud (p.H. 8.6) from a stream at Scotty's Castle in Death Valley, elevation 3000 feet; in water from a lily pond at the Huntington Library and Botanic Gardens, at San Marino, California; in water from a roadside irrigation ditch, five miles south of Isabella, California; twice in Sequoia National Park, once from a roadside stream (p.H. 6.9) seven miles below Giant Forest, and once from the Kaweah River (p.H. 7.4), near the Park Headquarters; and from a streamlet one-half mile west of Lake Arrowhead Village, elevation 5250 feet, in the San Bernardino Mountains.

Saprolegnia ferax (Gruith.) Thuret.

This species in California has proved itself a very interesting and at times a baffling one, thereby almost precluding identification. The plant is undoubtedly *S. ferax*, but variation makes a new description necessary. There are two forms of the species. Form 1 may be described as follows:

The very delicate and transparent primary mycelium, from halved hempseed, reaching a diameter growth of 32 mm. within 21 hours, being rapidly overtaken by the more prominent secondary hyphae, the latter reaching to 15–18 mm. within two days, after which the growth may slacken, although colonies up to 35 mm. are not uncommon. Primary sporangia appearing after twelve hours, somewhat cylindrical, barrel-shaped, or spindle-form; small, 80–120μ in length; generally terminating the delicate primary hyphae or borne on short, lateral branches, sometimes intercalary, in which case they empty their spores through a lateral branch. Secondary hyphae at first slender, sparingly branched, and wavy in young cultures, with hyaline tips; mycelium in older cultures transparent or opaque, commonly matted. Secondary sporangia (from permanent hyphae) more or less cylindrical, slightly broader basally or toward the middle and tapering distally, or occasionally otherwise; commonly 490–768 x 35.36–54.08μ, or as small as 216 x 24μ; hyphae renewed by internal proliferation to produce new sporangia beyond old ones, or sporangia may be formed immediately below the former basipetally, or at tips of new hyphae by lateral proliferation, as in *Achlya*. Zoospores sometimes very sluggish upon emergence, slowly swimming away, or at other times more rapidly discharged, pip-shaped or more commonly long and obliquely reniform, spherical at rest, mostly 10.4μ; germinating readily in water, often to form a delicate mat in the parent mycelium. Gemmae very numerous after two days, commonly elongated and of the same diameter as the hyphae or somewhat enlarged, often sporangia-like, frequently oval, ovate, obovate, or irregularly lobed; single or in chains, seldom clustered. Sporangia transformed from gemmae very irregular, often with as many as five long papillate branches, through one of which spores may discharge. Oogonia abundant after one week, usually terminal at the ends of main hyphae or on lateral branches, seldom intercalary; for the most part spherical, but often pyriform or sometimes elliptical, elongated ones not infrequently curved; 50–99μ in diameter, and in length up to 185.4μ for the more elongated ones; walls about 1.8μ thick; pits numerous, up to 10.8μ across; connecting hyphae sometimes disintegrating after the oospores have matured. Oospores 3 to 50, possibly more, commonly 9–25, not always filling the oognium, usually centric; the spherical ones 22.8–27μ, otherwise 18.22–21.6 x 22.8–34.2μ. Antheridia absent except in very rare cases.

Form II differs sufficiently from form I to merit a partially separate description. Macroscopic observations of the two molds are similar. (See plate I, figures 1–5.)

Sporangia from permanent hyphae abundant after three to five days, terminal or intercalary, the latter emptying by a lateral, papillate branch; sometimes partially constricted at intervals, often greatly branched and contorted, not uncommonly bulgy at places as the result of internal pressure from the crowding of spores against the very thin walls; up to 800μ long; proliferation often producing sporangia in clusters. Zoospores at rest somewhat variable in diameter, 10–23μ, mostly 10–12.6μ. Oogonia spherical, frequently with basal extension into stalk, at other times elliptical to elongate, or filiform and of same diameter as the hyphae, or they may be otherwise variously bulged or branched; the spherical oogonia up to 90–105μ in diameter and the filiform ones up to 264μ in length and approximately of the diameter of the connecting hyphae; walls about 1.8–2μ thick, with pits about 9μ across. Oospores two to many, commonly 17–20; 18–28.8μ, mostly 23.4–27μ. Antheridia absent.

The two above forms, with exceptions, conform fairly well to Minden's two types (Coker: The Saprolegniaceae, 1923, page 41). In both California forms, however, oogonia may be terminal on main hyphae, on short lateral branches, or even intercalary. Oogonial branches are frequently curved. All types of oogonia are found in both forms, but the elongated, cylindrical, or branched oogonia are more common in form II. Contrary to Minden's description, in the California form II, the cylindrical oogonia are not produced as a rule inside of empty sporangia but are freely independent, terminal or intercalary. Often a cylindrical oogonium is terminated by a spherical one, or there may be other combinations of two or three oogonia in a row. Oogonial pits in both types are sometimes broader than in the Chapel Hill form. In the filiform oogonia, the oospores form a single row.

Sporangia may often be formed immediately below old terminal ones, as has been recently pointed out by Wolf (1939), and there is great irregularity and distortion in the sporangia of later origin. In both California types, zoospores are commonly 10.8–12.6μ, but in form II they may often be as large as 18μ for non-discharged spores, once reaching a diameter of 23.4μ.

In the western types, the gemmae are very abundant, being so numerous as to give great opacity to the colony, whereas the Chapel Hill form (probably form II) is described as having "gemmae not greatly abundant."

Form I has been taken 75 times and form II, five times, from southern California, in both soil and water from many localities. Form I has been freely taken from Warm Creek, from Strawberry and Coldwater Canyons at Arrowhead Springs, from Devil Canyon, from the Santa Ana River bed, and from Horsethief Canyon, all near San Bernardino. Stations in the San Bernardino mountains include springs, tanks, and streams along the all-year road to Crestline, Lake Arrowhead, and Big Bear Lake, as well as Little San Gorgonio Creek, near Oak Glen; also from the South Fork Meadows (headwaters of the Santa Ana River) on the flanks of Mt. San Gorgonio, at an elevation of 8000 feet. In

the Sierra Madre Range, northwest of San Bernardino (west of Cajon Pass), form I has been secured from Big Rock Creek, near Valyermo, and from seepage water, one mile below Jackson Lake. It has been taken from Havilah Creek in Kern County, while in San Diego County the mold was procured from a dry stream bed in Live Oak (state) Park. Desert stations include small streams, dry stream beds, and springs, located in Death Valley National Monument (near Scotty's Castle), Chino Canyon near Palm Springs and White Water River (at bridge on U. S. Highway #99), and Quail Springs, in the Joshua Tree National Monument; also from a stream crossing on the Miller Canyon road, about five miles below Lake Gregory, on the desert side of the mountains, north of San Bernardino. Elevations of the stations range from about sea level to 8000 feet and the p.H. of the water samples from 6.2–8.6.

Form II has been isolated from Warm Creek in San Bernardino City, from Mill Creek Canyon at Sierradina, about one mile below Forest Home; and from the Santa Ana River; also from a boxed, stagnant spring on the Hesperia-Arrowhead road (desert side of the mountains), elevation 4500 feet, from the Kern River region, in Kern and Tulare Counties, and from a flower-conservatory pool in Balboa Park, San Diego.

The two molds have been in almost continuous cultivation (except for short storage periods through the summer months) since their first isolation, December 12, 1935, and April 22, 1938, respectively. Characteristic differences appear whenever the two forms are encountered.

Saprolegnia bernardensis n. sp.

Plate 3

Primary mycelium very lax and delicate, the hyphae transparent and very crooked, 12–15μ thick, giving rise within 24 hours to a few small sporangia. Sporangia generally more or less cylindrical to long barrel-shaped, straight or curved, seldom irregular, as a rule terminal; seldom more than 150μ long by 27.7μ thick. Secondary hyphae appearing on the second day and growing rapidly, reaching a colony diameter of 62 mm. on halved hempseed in water within one week, more extensive than in *S. ferax*; hyphae delicate, as a rule less than 12μ in diameter. Secondary sporangia scarce, similar to the primary ones in appearance but larger, up to 335μ long, possibly longer. Internal proliferation of hyphae common. Spores at rest spherical, 10.5–12.6μ. Gemmae not abundant; spherical, elliptical, pyriform, clavate, or elongate, sometimes branched, not very dense; terminal or intercalary, single or in short chains; a few becoming transformed into sporangia. Oogonia very numerous within five days, giving the more or less transparent colony a speckled appearance; borne commonly on short lateral stalks, seldom terminating main threads, sometimes clustered as the result of branching of the parent hyphae or of oogonial stalks, rarely proliferated from another oogonium, at times intercalary; spherical, oval, elliptical, clavate, to flask-shaped, frequently narrowed basally or apically into a long filament of the same diameter as the parent hypha; commonly measuring more than 300μ in length and even not infrequently over one millimeter, in one case reaching 1106μ; usually 41.4–97.2μ at greatest diameter (on cornmeal agar, 39.5–47.5μ); with greenish or yellowish tinged walls, 1.5–1.8μ thick, freely pitted, pits commonly about 9μ in diameter. Oospores 1–25 or more,

commonly 8–20 (1, 2 or 3 on cornmeal agar), not always filling the oogonium, centric; spherical, oval, or often in a single row and very long and narrow when found in the filamentous portion of an oogonium, such as 9.0 x 75.6μ, spherical ones 20–29.7μ, averaging 22.5–27μ. Antheridia generally present, at times androgynous, but more commonly diclinous; as a rule one to three, seldom more per oogonium, not completely covering the oogonium. Empty antheridia seen, germ tubes not observed.

The new species is more closely related to *S. litoralis* than to other *Saprolegnia* species, but differs sufficiently to be described as new. The habit of growth, the scarcity of sporangia, and the abundance of characteristic oogonia show similarity in the two species.

In hempseed-water culture, the oogonia of the new species show great variation, from small spherical ones containing one oospore to large ones with many (25 or more) oospores, and the oogonia are borne on short lateral stalks, of a length seldom more than twice the diameter of the oogonia. The elongated flask-shaped or clavate oogonia are conspicuously characteristic of the species, as a rule, such oogonia being intercalary. Oogonia are rarely terminal on main hyphae, as they occur in *S. litoralis*. Oogonia produced on cornmeal agar are generally smaller, more commonly spherical, 39.5–47.5μ in diameter, and bear usually one or two, rarely more than three large oospores. Such oogonia may commonly terminate the main hyphae.

As a rule, oospores are probably more than 25, whereas in *S. litoralis* they are commonly 2 to 6, but ranging up to 20. In *S. litoralis* the oospores are commonly 30–33μ in diameter, whereas in the new species the range is commonly 25.2–27.0μ, except for the peculiarly long ones described earlier, or when produced on agar, then approaching the size of those in *S. litoralis*.

Antheridia are more often diclinous from nearby hyphae, but they may be androgynous from near the oogonia and from oogonial stalks. Not uncommonly an oogonium may appear to rest on a nest of androgynous antheridial stalks, in which case the lower part of the oogonium may be completely invested by antheridia, these generally arising from the oogonial stalk. These characteristic antheridia suggest a kinship to *S. litoralis*.

The new mold, as described, was isolated from a cool stream, one-half mile west of Lake Arrowhead Village, in the San Bernardino Mountains, at an elevation of 5150 feet, July 30, 1940. Also taken on the same date from a small stream, near Twin Peaks P.O., elevation 5300 feet. Prior to that date the mold was taken a number of times but not identified: July 2, 1940, from milky, stagnant water taken from a U. S. forestry water-storage tank, on the Miller Canyon road, below Lake Gregory, elevation 2550 feet; July 23, 1940, from three of seven parallel streams, along with a new species of *Achlya*, in South Fork Meadows, elevation about 8000 feet, on the trail to Mt. San Gorgonio; and from Horseshoe Bend, July 20, 1940, near the type station, from dry sandy loam soil, 20 inches deep, on the floor of a pine forest, elevation 5500 feet. The mold appears to be identifiable with the higher elevations in the San Bernardino Mountains.

Saprolegnia species (new, non-fruiting, No. 1)

Plate 2, figures 1–5

Primary mycelium very delicate and freely branched. Primary sporangia occurring after one day, mostly terminal on short lateral branches, sometimes terminating main hyphae, rarely intercalary; more or less cylindrical, clavate, pyriform, oval, apiculate, or even spherical, and often irregular; small, commonly 40–96 x 24–40μ. Permanent mycelium of relatively slow but steady growth, reaching a diameter of 13 to 17 mm. within one week and growing little after that; colony at first clear-cut and transparent, later becoming paler and more opaque, as well as somewhat thicker vertically; hyphae variously branched or unbranched, crooked, often matting, frequently empty after one week, practically of same diameter throughout, 24–40μ, commonly ending in sporangia or gemmae. Secondary sporangia abundant within two to four days, generally long and slightly broader basally or toward the middle, as in typical *Saprolegnia* species, or tapering distally into a long filament, often narrow for their full length, frequently freely branched, in which case the branches may be short or long and characteristically filiform, one or more of these being terminated by a pore; the sporangia may also show characteristic bulges; 192–422 x 18–49μ; lateral branches commonly 45μ to over 150μ long. Spores upon discharge, behaving as in typical *Saprolegnia* species, except for their momentary clustering at the place of discharge and then slowly swimming away, many coming to rest soon against nearby hyphae; upon discharge generally about one and one-half to two times as long as broad, and somewhat obliquely reniform; at rest usually 10.8–12.6μ, although varying considerably up to 18μ, and occasionally larger in the case of nondischargeable spores, as 41.4 x 18μ; sporangia, especially when transformed from gemmae, not infrequently retaining from one-third to one-half of their spores, these disintegrating in place, rarely germinating there. Masses of floating spores numerous. Gemmae very profuse within one week, often becoming so plentiful after two weeks as to obscure all colony details, the culture building itself up vertically and often shrinking laterally, especially during the cooler winter months; spherical, elliptical, pyriform, clavate, or elongate and often tapered, the tapered ones commonly single and the others in chains up to twelve, both individuals and chains being occasionally branched; variable in size from 63μ to over 300μ in length. No oogonia produced in any cultures to date.

Only at times is the vegetative growth of this mold so extensive as that of other *Saprolegnia* species. Although at times extensive and well matted, early becoming white and of speckled appearance, the mycelium is generally of smaller extent, often thickly matted vertically, and opaque, colonies frequently shrinking with age. In the latter case, it is generally difficult to distinguish characters, microscopically, except at the very edge of the colony.

Later sporangia may bear a basal papillate branch, through which spores are discharged, or they may bear several lateral, filiform branches, through one or several of which spores are emptied, or the sporangia may be irregularly and prostrately branched. Many sporangia, especially the more irregular ones, may be very thin-walled, showing characteristic bulges where spores press against the walls. This last character may be found also in *S. ferax*, type II, but it is not nearly so common in that species.

No sexual reproduction has occurred to date in this mold, which was first

isolated April 23, 1937, from wet sand and fine gravel in Lytle Creek, at Highland Avenue, near San Bernardino. The mold has been in continuous cultivation since, all generations from a single spore. Various types of media have been tried, in order to induce sexual activity, none with positive results. Nutrient agar, corn-meal agar, carrot agar, hempseed, and buckwheat seed were those mainly used. Hydrogen ion ranges (pH) 4.5, 5.4, 6.4, 7.0, 7.6, 7.9, and 8.8 have produced no variable results other than differences in the extent of vegetative growth.

Additional non-fruiting strains of this mold, or a closely related one, have been procured from Oak Glen (elevation 5200 feet), and nearby localities, at Big Bear Lake (elevation 6750 feet), and from Rock Creek falls, in Mill Creek Canyon (elevation 4500 feet), all in San Bernardino County; from San Dieguito River, one mile below Hodges Dam, from Morettis, just below Henshaw Dam, and other points in San Diego County; also from the Kern River and its tributaries in Kern and Tulare Counties.

Although the above strains are closely similar, there are constant dissimilarities, which tend to place the molds in at least two groups. However, details of the two groups are being withheld until further studies are made, and a single description is given above based on a pure culture of a single isolate (Type station #2110).

Subsequent isolations have shown this mold to be one of the most common of the Saprolegnias, from all types of localities at varied elevations.

Dozens of sexual matings have been attempted in order to produce sexual reproduction, the last series of crosses and intercrosses involving 30 reciprocal matings, January 31, 1940, but only negative results have been forthcoming. The writer has had as many as a dozen isolates of this mold growing at one time.

Saprolegnia species (new, non-fruiting No. 2)

Plate 2, figures 6–15

Primary mycelium profuse, up to 15 mm. within 18 hours; hyphae small, very delicate, and transparent, with numerous denser sporangia-like bodies. Primary sporangia up to 250μ in length, usually shorter, formed within one day, terminal or intercalary, commonly cylindrical to filiform, though often pyriform, clavate, or even oval to elliptical, and not uncommonly bearing one or two side branches which may be as long as or longer than the sporangium proper, through which branches the spores may be released. Permanent mycelium stouter and more dense, opaque and somewhat pale at about two weeks, but later more transparent; hyphae at first straight, radiating prominently from the hempseed substratum, but later becoming branched and matted. Secondary sporangia appearing by the second day, first ones being long and broadest at the middle, below the middle, or beyond the middle, suggesting those of both *Saprolegnia* and *Achlya* species, later commonly with side branches or often bulging irregularly; up to 584μ in length, in case of those measured. Spores discharged through a pore terminating the main sporangium or a lateral branch, and immediately swimming away upon release; diplanetic; at rest 10.8–12.6μ in diameter. Gemmae very abundant within a few days, varying greatly as the colony grows older and with frequent changes of the medium; mainly at first long club-

shaped, then with few to many lateral branches which may arise at nearly right angles commonly from one side, the body of the gemma in that case bending toward the branch-free side, often variously constricted or narrowed, sometimes very irregular, and showing characteristic appendages; becoming freely transformed into sporangia, so that in old cultures practically nothing but empty sporangia, and wholly empty hyphae may be seen; commonly up to 400μ in length, with branches up to 600μ or more. Oogonia absent.

This mold may be characterized by its peculiar gemmae, which are quite different from any thus far described in the Saprolegniales. A hyphal tip destined to become a gemma thickens and becomes darker. Protuberances then begin to appear typically on one side, often on both sides or vertically. These protuberances may rapidly grow into short, thick branches, the branches assuming the proportions of additional gemmae, these being as large as or larger than the parent hypha. Also they may show constrictions and alternate inflations, and may be often greatly narrowed distally.

Laterally placed gemmae are strikingly characteristic, suggesting a deformed hand or an enlarged basidium with its sterigmata or they may be otherwise very irregular, especially in the middle third of the circular mycelial mat in a week-old hempseed-water culture. A change of medium, such as adding fresh water to a culture, may cause gemmae to germinate, producing small hyphae, which often cause a delicate mat to be formed in the parent mycelium. Gemmae are freely transformed into sporangia. New gemmae develop freely beyond old ones, which in turn may sporulate. In an older culture (three weeks) nearly all gemmae and hyphae may be empty except for a few recently formed ones, thus making the colony appear almost wholly transparent.

This plant has been found six times to date, it being secured first in a water sample, January 4, 1938, from the San Jacinto River near its point of entrance to Lake Elsinore. It was found again May 6, 1939, in coarse sand and water taken from Pauma Creek in San Diego County, at an elevation of 720 feet. It was found also in a water sample from the upper end of Big Bear Lake, elevation 6750 feet, May 28, 1939. Additional specimens have been taken from Pitt River, mud and water, near Canby, Modoc County; from Howard's Gulch, also near Canby; and most recently in San Bernardino, after a heavy rain, from wet sand and silt, lodged against a curb at Grant and Scott Streets, near the junior college.

Matings of all of the above strains with the original type, No. 2169, as well as with the preceding type, No. 2110, gave no positive sexual response.

ACHLYA

Achlya species have been taken from a wide area. These include five new species, *A. pacifica*, *A. pinnulata*, *A. diffusa*, *A. californica*, and *A. heteromorpha*, as well as *A. caroliniana* Coker, the last locally plentiful, and several fruiting and non-fruiting forms, not yet identified.

Achlya caroliniana Coker

This species seems to be highly localized in southern California, it being found often in the canyon streams about Arrowhead Springs Hotel, elevations about

2000 feet, and from Devil Canyon, both near San Bernardino. The California type agrees in every respect with the original (Coker, 1923), except that in most strains antheridia are constantly present and generally declinous.

Achlya pacifica n. sp.

Plate 4, figures 1–7

Mycelium to 30 mm., over-all growth, on halved hempseed within one week and of limited growth after that, dense and more or less opaque, somewhat dull in old cultures. Individual hyphae radial and prominent in young cultures, generally about the same diameter throughout, commonly between 25 and 50μ. Sporangia typically long and irregularly cylindrical to barrel-shaped, or broader beyond the middle; generally terminal and single, sometimes otherwise; up to 648μ in length, occasionally longer, seldom less than 200μ long, 60–80μ wide at the broadest place; possessing one or more lateral as well as terminal papillae and pores, through all of which spores may be discharged; renewed by sympodial branching, the branches sometimes shortened in such a way as to produce terminal clusters of three or four sporangia. Spores upon discharge congregating at apex of sporangium or forming small masses near the lateral pores, or occasionally encysting within the sporangium, second swimming stage frequently absent; spherical, 10.8–11μ at rest, one measuring 18μ within the sporangium. Gemmae dense, numerous, 5 to 8 commonly in a filament, often more, gemmae and filaments sometimes freely branched; 80 to over 200μ long. Oogonia abundant after three days, terminating small lateral branches or developing as intercalary units in the hyphae; lateral ones more or less spherical, 41.4–72μ, intercalary ones often several in a row, with single oogonia measuring up to 135μ in length and 45–50μ in breadth; walls about 1.5μ thick, with yellowish tinge, unpitted, smooth or at times bearing numerous germ-tube-like projections. Oospores most commonly 1 to 5, often up to nine in the spherical oogonia or to 25 or more in the intercalary oogonia; spherical or compressed to elongated when crowded; 18.2–34.2μ, averaging 23.4–25.2μ; eccentric, with one large lateral oil droplet; wall about 1.5μ thick. Antheridia present on many oogonia, although many cultures show none; nearly always diclinous; produced on long and greatly coiled or gnarled, irregular stalks.

This mold shows similarities to both *A. flagellata* and *A. caroliniana*, there being sufficient contrast to warrant the establishment of a new species. In many respects the habit of growth more nearly resembles that of *A. caroliniana*. Hyphae are not so thick, 25–50μ, at maturity as in *A. flagellata*, in which species the hyphae may often approach 100μ in thickness.

In general the sporangia are larger than those of *A. caroliniana* but characters suggest relationship to that species. After discharge, spore masses may break away from sporangia at the point of discharge and float about in the culture until they decay, or segregated spores may germinate on the bottom of the culture dish. Frequently some of the spores may remain in the sporangium, some of them germinating there.

The oogonial habit of the new plant has characteristics intermediate to *A. flagellata* and *A. caroliniana*, but with respect to position more closely resembling *A. caroliniana*. In size the spherical oogonia are nearest those of *A. flagellata*. Although the mold had been in continuous cultivation for nearly eighteen months after its isolation, the intercalary oogonia appeared for the first time,

suddenly and very abundantly, in a water-hemp culture, December, 1939. These oogonia differ from those of *A. caroliniana* in being larger and in containing more oospores. A filament of such oogonia may reach a total length of nearly one millimeter. Oogonial projections when present are prominent and more numerous than in *A. caroliniana*.

Oospores in *A. flagellata* range in number from one to ten, commonly two to six, whereas in *A. caroliniana* there are one to two, rarely four. In the new mold they range from one to nine, commonly three to five, although frequently there is a single large one. In size the oospores more nearly agree with those of *A. caroliniana*, but not wholly so. In *A. caroliniana* they measure 18.5–23μ, averaging 22μ, in *A. flagellata* 26–35μ, averaging about 28μ. In the new mold they average 23.4–25.2μ but single oospores may range up to 34.2μ. Occasionally oospores may be considerably elongated in the intercalary oogonia, one such oospore measuring 14.4 x 41.4μ.

Antheridia when present are nearly always diclinous, often terminating gnarled, slender stalks. Coker and Braxton (1926) describe a strain of *A. caroliniana* with antheridia on 25–40% of the oogonia; otherwise this species may not possess antheridia. The new plant is not *A. caroliniana*, because the latter species, both with and without antheridia, has been isolated several times locally and shows sufficient differences.

Gemmae, as well as intercalary oogonia, after a few days very frequently bear numerous small, germ-tube-like branches, a characteristic described by Coker (1923) as occurring in *A. klebsiana*.

To date three isolations of this species have been made. The type station for the new mold is wet sand from a stream through the golf course, near Santa Fé Rancho, California (elevation about 75 feet), on the road to Oceanside; collected April 3, 1938. Found also in dry sand from the Santa Ana River at Sterling Avenue bridge, near San Bernardino, and again in moist sandy silt, 8 inches deep, from a dry drainage ditch leading to Little San Gorgonio Creek, near Oak Glen, elevation 4500 feet.

Achlya pinnulata n. sp.

Plate 4, figure 8, Plate 5, Plate 7, figure 1

Colony growth on halved hempseed up to 20–22 mm. within a week and of limited growth thereafter, after one week becoming denser, matted, opaque, and pale, occasionally to almost black in old age. Sporangia more or less cylindrical, or broader basally or near the middle, early ones (after one day) commonly very small, 128 x 52μ, often quite long and narrow, 1080 x 48μ in one case, thin-walled, and often greatly distorted and wrinkled after spores are released, not uncommonly emptying by several mouths or papillate branches, such branches often up to 160μ or more in length. Proliferation of new hyphae occurring as in other Achlyas, by sympodial (alternate) branching or very commonly by opposite or even occasionally by whorled branching, these in turn being likewise renewed; sporangia often produced in clusters. Spores dischargeable by one or several pores to congregate at points of discharge, many of them remaining permanently at those points while others may be diplanetic in their behavior, but frequently some or all spores remaining undischarged, these often germinat-

ing in place; discharged spores at rest 10.8–12.6μ, undischarged ones occasionally up to 19.8μ. Gemmae formed by segmentation of main hyphae, at times not very numerous, similar to sporangia and generally in terminal position. Oogonia appearing by the second day, often so abundant within one week, along with the parent hyphae, antheridia, and antheridial stalks, as to obscure colony details; borne singly on lateral stalks or not infrequently two or three oogonia from the same stalk; for the most part spherical, often narrowed basally to diameter of the supporting stalk, or seldom comprizing part of the main hypha, rarely irregularly lobed with spherical bulges in such a way as to suggest a cluster of grapefruit, with each lobe enclosing one or two oospores; spherical ones commonly 54–63μ, often larger; walls less than 2μ thick, seldom pitted except where antheridia touch. Oospores eccentric; numerous, 4 to 6 for the smaller oogonia and up to a dozen or more, seldom as many as 35, for the larger oogonia; spherical or oval when compressed, mostly 21.6–23.6μ in diameter, sometimes larger. Antheridia commonly many to each oogonium, usually diclinous, but frequently androgynous from a distance, or antheridial stalks often arising from one oogonial stalk to be applied to an oogonium elsewhere, often at considerable distance.

The occasional appearance of a characteristically lobed oogonium distinguishes this mold from all others. Such an oogonium may possess as many as six or eight almost spherical lobes, the whole unit suggesting a cluster of grapefruit, with each lobe bearing one or two oospores of normal size. Possibly at times such a unit is composed of many small oogonia. Very rarely may a moniliform or a club-shaped oogonium be formed.

Antheridia are generally several per oogonium, although seldom numerous enough or sufficiently contorted to obscure details of oospores. A single diclinous stalk may produce two or more antheridia and often there are several stalks present. Androgynous antheridial hyphae may arise more than a millimeter from an oogonium, the crooked thread ultimately reaching the oogonium.

This new species was originally isolated from a sample of wet sandy soil, from San Diego County, given to the author by Dr. O. A. Plunkett, of the University of California at Los Angeles, April 14, 1939. Other isolations include soil from a roadside ditch, east side of Scott Valley, near Etna, Siskiyou County, in northern California; twice from water and soil from a spring and a stream in Howard's Gulch, near Canby, California; and beach sand-silt from the North fork of Kern River at Onyx Ranch, near Isabella, California.

Achlya diffusa n. sp.

Plate 6, figures 4–8

Colony growth on halved hempseed in water normally about 25–27 mm. in diameter within one week and seldom larger thereafter, the transparent and very delicate mycelium soon displaying speckled white masses of oogonia and resting bodies; the crooked, sparingly branched hyphae not readily distinguishable to the naked eye, generally 25–40μ in diameter basally, the colony becoming pale and diffuse in old age. Sporangia in both young and old cultures scarce; smaller than in most *Achlya* species, in the few cases observed 140–418 x 20–28μ; cylindrical, of approximately the same diameter as the hyphae, and emptying by a terminal papilla. Occasionally a few small masses of spores and rarely a swimming spore seen in culture. True gemmae, when present, terminal or otherwise,

produced singly or in chains of generally fewer than six, longer chains being uncommon; when terminal, shaped like sporangia, subterminal ones cylindrical or only slightly larger than the parent hypha, straight or curved, often very long and filiform, not freely separating at maturity; becoming transformed into sporangia or sprouting by very numerous germ tubes. Oogonia very abundant within a few days, borne usually on lateral short stalks, as a rule not much greater in length than the diameter of the oogonia, frequently two oogonia per stalk, very freely proliferating, with the newer body receiving the contents of the older; for the most part spherical, sometimes narrowed basally, sometimes otherwise, as long flask-shaped; commonly 72–109.8μ in diameter, although smaller (57.6 x 63.0μ) or larger ones (180μ in length) have been seen; wall 1.8 to 2.0μ thick, with a greenish or yellowish tinge, pits generally absent, though occasionally present and very numerous. With respect to size, shape, and degree of abortion, oospores variable even in a single oogonium; 1 to 13, sometimes more, not completely filling the oogonium, frequently 1 or 2 in larger, older oogonia, others having aborted; 14.4–28.8μ (mostly 21.7–28.8μ) or even larger, up to 54μ for one spherical oospore and 56.7 x 48.8μ for an oval one; eccentric with one large oil droplet or at times with several smaller scattered droplets; walls 1.3–1.5μ thick or at times thicker. Antheridia always present, one to several per oogonium, these with their stalks often completely covering the oogonium, always declinous, commonly applied to oogonium by blunt foot-like processes. Definite germ tubes and empty antheridia readily observable.

This plant was isolated several times from the same locality, from eight parallel streams, varying from a few feet to about 50 feet apart, in South Fork Meadows (South Fork of Santa Ana River), in the San Bernardino Mountains, at an elevation of approximately 8000 feet. One other water mold, *Saprolegnia bernardensis*, was taken freely with this form, as well as possibly *Geolegnia* species, which was eliminated because of bacterial contamination.

An outstanding feature of this species lies in its extensive proliferation of oogonia, and the production of large, spherical or balloon-like bodies. Repeated proliferation may produce long chains of incompletely developed oogonia-like bodies, up to eight in one case observed, or two or three such bodies may appear as outgrowths from a single one. Mature oospores are produced more commonly in the oogonia that do not so freely proliferate. One balloon-like oogonium measured 165.9 x 118.5μ and contained 23 incompletely developed oospores, although as a rule these bodies are empty and only remotely resemble oogonia. Such bodies may reach a length of 250μ.

With respect to the presence of inflated spherical bodies, the plant strongly resembles *A. inflata* Coker (1927). However, hyphae are probably thicker than in that species. Oogonial walls frequently are not pitted; the inflated oogonia-like bodies are never pitted, and the oogonial stalks are shorter than in *A. inflata*. Oospores are more numerous, often almost filling the oogonia, though at times scarce or entirely aborted, as in *A. inflata*. Oospores are smaller in the new plant, 14.4–28.8μ (mostly 20.7–28.8), although they may be occasionally larger, as 56.7 x 48.8μ, as contrasted with 29–37μ (averaging 31–35μ) for *A. inflata*. In his description of *A. inflata*, Coker (1927) could not trace the antheridial hyphae to their points of origin. In the new species there is easily traceable an abundance of antheridial hyphae, these being gnarled and crooked and freely branched, strongly suggesting the condition found in *A. proliferoides*.

The new mold differs from *A. proliferoides*, which species it also closely resembles, and from *A. flagellata* by the extent of growth, the growth in the new species being greatest. The hyphae at their base are not so thick as in *A. flagellata*, nor so irregular and wavy as in *A. proliferoides*. Oogonia are larger in the new species, commonly 72–109μ and up to 182μ, as compared with 40–55μ for those of *A. proliferoides* or 48–75μ and up to 100μ for *A. flagellata*. Also the oospores are larger than in *A. proliferoides*.

The extensive proliferative habit of oogonia strongly suggest that of *A. proliferoides*, and the fact that the oospores frequently go to pieces indicate a possible relationship. However, the apparently universal diclinous habit of the antheridia and the lack of coiling of antheridial branches about non-fruiting hyphae suggest a less close kinship.

Achlya californica n. sp.

Plate 6, figures 1–3, Plate 7, figure 2

Colony dense, prominent, occasionally producing considerable aerial mycelium above hempseed substratum in water, the colony reaching a diameter of 20–30 mm. within four days and of limited growth thereafter. Hyphae stout and tapering, or smaller and of uniform diameter throughout, very crooked; 23.7–118.5μ in diameter basally, more commonly 45–95μ; branched apically, usually to end in sporangia or gemmae. Sporangia never numerous, primary ones delicate and small, seldom larger than 250 x 50μ; those formed later little or no broader than the hyphae and gracefully tapering distally, not uncommonly extended into a short, narrow filiform tube containing a single row of spores; frequently bent or crooked; averaging 237–395μ in length, but occasionally more than 600μ, and 45–55μ broad; spores discharged through a terminal pore or by one or several lateral papillate branches, such branches being seldom longer than 50μ; spores upon discharge forming irregular floating masses which may or may not enter into a second swimming stage, or they may germinate directly, less frequently a few or all spores remaining in the sporangia to germinate there; 10.8–12.6μ at rest. Gemmae plentiful, formed as a rule from the smaller hyphae, and seldom over 55μ thick, single or in chains; terminal ones sporangia-like or filamentous and the subterminal ones cylindrical, often with rounded ends, oval, or club-shaped, tending at times to separate at maturity; 300–1000μ in length; becoming transformed into sporangia or germinating by means of a number of terminal or lateral germ tubes. Oogonia abundant, commonly hidden by the densely matted hyphae; spherical or oval to pyriform, occasionally otherwise; 36.0–90.0μ x 57.6–126μ, averaging 64.8–90 x 66–93.6μ, sessile or more often terminating lateral stalks which may reach a length of more than four times the diameter of the oogonia, rarely intercalary; seldom proliferating as in *A. proliferoides* but multiple oogonia formed at times on a common stalk, as in *A. caroliniana*; walls smooth or occasionally undulated or wavy in outline; 1.5–2.0μ thick, greenish or yellowish-green; pits when present 6.3–7.2μ, occasionally up to 9μ across. Oospores usually 1 to 9, sometimes as many as 15; varying in size even in the same oogonium; spherical, oval, ovoid to pyriform, or at times when single tending to assume the shape of the oogonium; 21.6–41.4 x 24–45μ, mostly 25.2–27μ for the spherical ones; eccentric with a large lateral oil droplet or with several scattered smaller droplets, or degenerating as in *A. conspicua*; walls 1.0–3.5μ thick. Antheridia always present, usually one, two or three per oogonium, androgynous from the oogonial stalk and from the parent hyphae, as well as diclinous; often applied to oogonia by blunt foot-

like processes, as in certain other *Achlya* species. Empty antheridia and germ tubes seen.

With respect to the prominence of the mycelium, the new mold somewhat resembles *A. conspicua*. In that species the hyphae are 30–166μ in diameter, more commonly 50–94μ, and in the new form the hyphal thickness ranges 23.7–118.5μ, more commonly 45–55μ, thus on the whole being smaller. The hyphal tips in the new mold do not wither, being replaced by newly proliferated growth, as described for *A. conspicua*. In *A. conspicua* sporangia are numerous, whereas in the new species they are scarce. On the other hand, gemmae are plentiful in the new mold. In size, oogonia somewhat agree with those of *A. conspicua*, but in the new species the walls for the most part are unpitted, though in an occasional oogonium the pits are very numerous. The oogonial stalks do not have a tendency to flare in the new plant. Oospores are of about the same size in the two species. When mature, oospores are typically eccentric in the new species, and they abort freely. Occasionally the oospore wall may be very thick, one such wall measuring 3.5μ. In the new mold, antheridia are both androgynous and diclinous; in *A. conspicua* they are reported as androgynous, rarely diclinous. The new mold suggests *A. americana* with respect to antheridial habit, but otherwise it is entirely different.

The type specimen was isolated July 2, 1940, from Waterman Creek, at the first bridge above the Arrowhead Arch, on the Waterman Canyon Road, north of San Bernardino, at an elevation of about 2500 feet. The mold has also been taken from Warm Creek at the South "E" Street bridge and from the Santa Ana River at South "E" Street, near San Bernardino, as well as from two stations near Oak Glen, in the San Bernardino Mountains, elevation 4200 feet, dry coarse ditch sand; again from a small swift irrigation stream at the Parrish Ranch, below Oak Glen, elevation 4500 feet, and from the pool in the plant conservatory in Balboa Park, San Diego.

Achlya heteromorpha n. sp.

Plate 6, figures 3–7

During the period of time allotted to the present study, the author has isolated numerous cultures of non-fruiting Achlyas, which have been narrowed down to four strains. In attempted reciprocal sexual crosses made October 4, 1939, two of the strains produced sexual bodies within five days, by October 9,1939. Sexual bodies are produced plentifully both in hempseed-water culture and on corn-meal agar. The two strains are very dissimilar, and through necessity have to be described separately.

STRAIN "A" (Station #2190):

Mycelium up to 35–37 mm. (diameter growth) within ten days; hyphae prominent to the naked eye, dense and white; generally about 50μ thick, although they may reach 100μ or more, and of practically the same diameter throughout; straight or crooked, branched. Sporangia appearing by the third day and very abundant within one week, terminating practically all hyphae, also subterminal

or intercalary; more or less cylindrical, or broader at the middle or below the middle, and gracefully tapered distally; seldom with lateral branches, such branches when present arising commonly from subterminal or intercalary sporangia; up to 960μ long, and 56–104μ at the greatest diameter; hyphae renewed by sympodial branching, often in such a manner as to produce clusters of three or four sporangia. Spores discharged through apical or lateral papillae, forming a hollow cluster at the point of discharge, these clusters seldom becoming detached even in old cultures except when disturbed, and the discharged spores uncommonly disintegrating in place; at rest 10.8–12.6μ. Gemmae plentiful within ten days; for the most part long and more or less cylindrical, or they may be somewhat ten-pin or flask-shaped, and in some cultures spherical, single or in filaments up to twelve for the elongated ones, and the spherical ones single or in chains of two or three, dense in young cultures, freely breaking apart in old cultures; the more cylindrical ones measuring up to 360μ long and generally 50–60μ in diameter, the spherical ones about 200μ, suggesting immature oogonia. No oogonia produced to date, unless crossed with strain "B."

STRAIN "B" (Station #2361):

Mycelium up to 30 mm. (diameter growth) from hempseed substratum within ten days; individual hyphae very large, white and prominent (more so than in strain "A"), 30–100μ in diameter, gracefully tapered and pointed at outer ends, and freely branched, especially in the outer half of the colony. Sporangia scarce, the terminal ones, when present, tapered distally, and intercalary ones long and slender, one of the latter type measuring 1600 x 45μ. A few free spores seen, swimming or floating, and some germinating in water culture. Gemmae abundant, borne singly and terminally or produced in a row by hyphal segmentation, often tapered when terminal, otherwise more or less cylindrical, commonly with rounded ends, and frequently with laterally placed terminal, germ-tube-like appendages, or otherwise branched, often partially separating and maturing, black; some measuring more than 1000μ in length, others much shorter. No sexual reproduction occurring to date, unless crossed with strain "A."

In mating "A" and "B" strains:

Oogonia plentiful within three to five days on both cornmeal agar and halved hempseed in water; spherical, oval or pyriform, terminating lateral branches, or filamentous and intercalary; spherical oogonia commonly 54–68.4μ in diameter, seldom larger, intercalary ones up to 195μ in length and of the diameter of the hyphae bearing them, 18–30μ; walls unpitted, except where antheridia touch, smooth, about 1.3μ thick, and with a greenish tinge. Oogonial stalks generally longer than the diameter of the oogonia, though sometimes shorter, frequently reaching more than 1000μ; as a rule very narrow basally, 9–18μ across, and commonly broadened distally, to 27–40μ, at point of attachment of oogonium. Oospores commonly 3 to 9, sometimes more, up to 21 in one case, spherical or sometimes oval to elongate when produced in a row in the filamentous oogonia, otherwise not completely filling the oogonia, eccentric; 18–23.4μ, seldom smaller or larger, one elongated oospore measuring 25.2 x 12.6μ; walls about 1μ thick. Antheridia very numerous on all oogonia, with their stalks frequently greatly coiled about oogonia and hyphae, there being many greatly branched and gnarled antheridial hyphae approaching each oogonium; fertilization tubes not seen.

Macroscopically, the extent of growth of the two strains is about the same, but the hyphae of the male strain are more prominent, dense, white, and coarser.

In the outer zone, whorls of short branches are very evident, especially in the male strain, these branches for the most part, as well as the main hyphae, having been transformed into gemmae. The male strain has rarely produced sporangia to date, while the female strain has produced a very great quantity of sporangia. Masses of spores in the more delicate mycelium of the female strain give a finely speckled appearance to the naked eye. Spores sprout freely in the liquid medium, less freely in agar.

Microscopically, the female strain agrees in many respects with *Achlya bisexualis* Coker (1927). Sporangia are essentially alike in both species but the spores are larger in the newer species (10.8–12.6μ, as contrasted with 9.6–10.8μ). Gemmal habits may closely resemble those of *A. ambisexualis* Raper (1939), except for the abundance of perfectly spherical gemmae in the new species, these consistently measuring about 200μ in diameter. In the male strain, gemmae are more abundant and they may reach hundreds of microns in length, often between 1000 and 1500μ, or even more. The gemmae may be smooth and cylindrical or they may show bulgy undulations. There may also be present laterally placed terminal branches, these at first suggesting germ tube growths.

Sexually, the new species may suggest habits of *A. bisexualis* and *A. ambisexualis*. Oogonia are produced individually at the end of lateral stalks which may vary in length from slightly less than the diameter of the oogonia to many times as long. In the new species, the oogonial stalks may be further characterized by their gradual increase in thickness distally, these at times being three times as great in diameter at the apex as at the base, especially for the longer stalks. On the whole, the spherical oogonia are smaller, 54–68.4μ, than the average for *A. ambisexualis*, 65–85μ, more nearly agreeing possibly with *A. bisexualis*, 50–80μ. Oospores are fewer in number, three to nine, than the average, eight to fourteen, for *A. ambisexualis*; in *A. bisexualis* the range is two to ten. The oospores are smallest in the new mold, 18–21.6μ, as contrasted with 20–27μ (22–24μ) in *A. ambisexualis* and 24–30μ in *A. bisexualis*. No intercalary oogonia have been described for *A. bisexualis*, although they are described for *A. ambisexualis* var. *gracilis* Raper (1939).

The female strain has been obtained plentifully in certain localities, especially locally, the first isolate being made on January 23, 1938, along with *Saprolegnia diclina*, from water and sand taken from Warm Creek at Sixth Street bridge, in San Bernardino; again on April 23, 1939, from muddy water taken from a ditch at Arrowhead Avenue, near the National Orange Show, about a mile below the first station. The mold has been taken in nearly all subsequent collections from Warm Creek for its entire length, five miles. (At its point of origin, the creek water has a temperature above 40°C.) Other local isolations include a lily pool at the Loma Linda Sanitarium, water and soil from San Timoteo Creek at South Waterman Avenue, from a stream near Seventh Street and Waterman Avenue, and from the Santa Ana River at South "E" Street. The mold has been taken twice from San Juan Creek: once at San Juan Capistrano, on U. S. Highway #101, at bridge No. 55-06, and again three miles from San Juan Capistrano, on the Ortega state highway; also from San Mateo Creek, north of

San Diego, U. S. Highway #101; from Flynn Springs (wet sand in a stream bed), east of San Diego, U. S. Highway #80; and from a small creek near Escondido. The most interesting isolation was made from coarse moist sand, in a small dry irrigation trench, at Furnace Creek Ranch in Death Valley, elevation −178 feet. The p.H. range for the habitats of the female strain have been 6.2–7.4. The type male strain was isolated from a sample of sand, silt, and water, taken from the south fork of the Kern River, at Isabella, California, April 29, 1939. This strain has been taken once since, from Daley Creek (water at p.H. 6.2), near Blue Jay, in the San Bernardino Mountains, elevation 5250 feet.

OTHER WATERMOLDS

In addition to the numerous species of *Saprolegnia* and *Achlya*, non-fruiting species of *Dictyuchus* and *Aphanomyces* have been very abundantly isolated; other watermold species have been less evident. A fruiting strain of *Dictyuchus* has been taken once; *Aphanomyces balboensis*, n. sp., twice from the same station; *A. laevis*, four times; *A. scaber*, twice; *Brevilegnia megasperma*, twice; *Calyptralegnia unisperma* var. *litoralis*, twice; *Geolegnia inflata*, once; *G. septisporangia*, twice; and *Pythiopsis*, a non-fruiting species, five times. *Allomyces* species have appeared five times. *Olpidiopsis Saprolegniae*, parasitizing *Saprolegnia*, has been found a few times; accurate data were not kept as to its frequency of appearance.

Aphanomyces balboensis n. sp.

Plate 8, figures 1–7

Hyphae delicate, straight or sometimes wavy, especially toward the distal end, rounded on end, little branched, about 4–8μ in diameter; whole hyphae readily transformed into sporangia; spores in sporangium short rod-shaped, upon discharge forming a rounded mass at mouth of sporangium, the mass often breaking away to float up against other hyphae; spores 9μ in diameter upon encystment. Oogonia terminal on lateral stalks, which may be from less than one to about twice as long as the diameter of the oogonia; usually spherical, sometimes slightly oval, occasionally irregular; 20.8–27.2μ. Oospores one, spherical, eccentric; 13.6–16.8μ, wall 0.5–0.8μ thick. One antheridium usually present, usually about 20μ in length, diclinous from a distant hypha or arising from one near the oogonium, seldom androgynous. Antheridial stalks generally about 3.2μ thick.

The plant was at first classified by the writer as *A. laevis* deBary, on account of its generally spherical or subspherical, smooth-walled oogonia. However, the occasional presence of irregular oogonia and smaller sized oospores, 13.6–16.8μ, contrasted with 16.5–26μ for those of *A. laevis*, brought about further study. The entire absence of antheridia or the presence of a single, large antheridium instead of several, and the non-wrapping of the antheridial stalks about the oogonia definitely removed the plant from *A. laevis*. Possibly antheridia may be also androgynous from oogonial stalks.

The plant was collected in two water samples from the penguin pond at the San Diego Zoo, Balboa Park, San Diego, June 23, 1938, at which time sketches were made.

Aphanomyces laevis deBary

This species, which agrees in every respect with the description given in Coker's book (1923), has been procured four times from the 604 collections in Southern California. It was twice isolated from Warm Creek, at the South "E" Street bridge, in San Bernardino, November 30, 1936, once from a water sample and once from sand nearby. The mold has not been taken locally since that date. The third and fourth isolations have been from widely separated stations: along with non-fruiting species of *Aphanomyces* and *Dictyuchus*, from cold water in Dollar Lake, p.H. 7.0, elevation 9300 feet, on the west flank of Mt. San Gorgonio, July 23, 1940; and from water and coarse sand taken from the Sweetwater River, San Diego County, State Highway #79, near Cuyamaca Lake, underneath bridge No. 57–56, August 18, 1940.

Aphanomyces scaber deBary

This species has been taken twice from soil: once from a rose garden at the author's home, in San Bernardino, in the upper one inch of moist black soil; again from sandy mud, deposited by rain run-off, at the northwest corner (gutter) of Richardson and Scott Streets, at the junior college athletic field.

Aphanomyces species (non-fruiting)

Non-fruiting cultures of *Aphanomyces*, apparently all of the same species, have appeared 52 times in 604 collections from southern California, generally from water samples, at a great range of p.H., as 6.6–8.8, seldom from soil. It has been secured from every county in southern California where collections have been made, at elevations ranging from slightly above sea level in Death Valley to 9300 feet at Dollar Lake on the west flank of Mount San Gorgonio. In some localities, where this plant has been taken, no other watermolds have been obtainable to date.

Attempts at sexual matings have produced no positive results. It may be pointed out here that even in fruiting specimens oogonia are scarce, seldom being observable outside of the original culture.

Dictyuchus species (fruiting)

Plate 8, figures 8–11

This species is somewhat similar to a fruiting species which the writer identified as *D. Magnusii* at Madison, Wisconsin, in the summer of 1927, from soil collected at Clinton, Mississippi, June 14, 1927. At that time oogonia were produced suddenly and abundantly when the colony rested on small clear quartz sand grains in shallow water, then never again. It also closely resembles an unnamed fruiting species described by Coker and Braxton (1926).

Sporangia and spores as described for *Dictyuchus sterile* Coker (1923). Oogonia, when present, terminating slender hyphae which are usually 5 to 10μ thick; spherical, or subspherical to pyriform; 39.6–45.0 x 37.8–38.6μ; wall more than 1.5μ thick. Oospores one, spherical; 28.8–30.6μ; eccentric, with a large

lateral oil droplet measuring 20–21.6μ; wall less than 1.5μ thick. Antheridia numerous, commonly three, and with their stalks wrapped about the oogonia; highly diclinous; 12.6 to 18μ or more in length; stalk slender, crooked, about 5μ in diameter.

Fruiting occurred only once in an otherwise non-fruiting strain, which had been in cultivation from the date of its collection. The species was obtained from dry soil taken in Balboa Park, San Diego, June 23, 1938. Numerous oogonia appeared November 1, 1938; since that date no other oogonia have appeared. The culture later became contaminated and had to be discarded.

Dictyuchus species (non-fruiting)

There appear to be no differences between the sporangia and hyphae of the non-fruiting and fruiting strains, except in rare cases, when the sporangial habit closely suggests that of *Brevilegnia* or *Thraustotheca*, in which case the agar growth is not exactly the same as in the "standard" type.

These non-fruiting colonies have been abundantly procured from all regions in southern California. In northern or central California, where collections have been made, *Dictyuchus* has at times been the only mold isolated. It has been taken 68 times in 650 collections. With rare exceptions, referred to above, the isolates seem to belong to the same species, and it has been one of the most common of all Saprolegniaceous molds. Isolations have been made at varied elevations, 1100 feet in San Bernardino to 9300 feet at Dollar Lake in the San Bernardino Mountains.

Although many attempts at sexual mating of like and unlike forms have been tried, in order to procure sexual reproduction, no positive results have been obtainable.

Brevilegnia megasperma Harvey

This species is exactly the same as the author's original type (1930), taken September 3, 1927, in New York State. Found twice in California, once from moist soil from the triangle at the old intersection of south "E" Street and Colton Avenue, at the Santa Ana River Bridge (elevation about 1100 feet), near San Bernardino, and again near Idyllwild, on the southeast flank of Mount San Jacinto, from a stream, at the junction of the Idyllwild and the Pines to Palms highway (elevation 4430 feet).

Calyptralegnia (Thraustotheca) unisperma var. **litoralis C. & B.**

This species agrees exactly with that described by Coker and Braxton (1926) and reclassified by Coker (1927). This plant has been isolated twice from all western collections. It was first obtained from black, dry soil, around the roots of *Juncus* species, 7 inches deep, from Horse Meadows, in the San Bernardino Mountains, elevation about 7500 feet, on the trail to Mt. San Gorgonio. The second isolation was made from black mud and cold water, taken from a marsh near Big Pines recreation camp, in the Sierra Madre Range, at an elevation of 6000 feet, on the old Big Pines highway.

Geolegnia inflata Coker and Harvey

This interesting species appeared one time among all collections made. The culture, never purified, was finally lost as the result of contamination. The species agreed in all respects with the original description (Harvey, 1925). The local type station may be described as #2078, April 11, 1937, gravelly sand, two inches deep, from a side road, at Blue Cut, U. S. Highway #66, near San Bernardino (elevation 2600 feet).

Geolegnia septisporangia Coker and Harvey

In all western collections to date, this species has appeared twice. The mold was taken from water and silty sand, p.H. 7.4, from Cedar Creek, four miles below Walker Pass, in Kern County, April 19, 1940. The second isolation was from warm water, from a drinking fountain, at Panorama Point on the Rim-of-the-World highway, near San Bernardino, at an elevation of 3800 feet.

Pythiopsis species

Non-fruiting species of *Pythiopsis* have been taken five times from widely scattered areas, but within the same mountain range: Coldwater Canyon, at Arrowhead Springs, elevation about 2000 feet, north of San Bernardino, from damp, coarse sand at the head of a small alluvial cone; Miller Canyon, stream across road, elevation about 3000 feet, about four miles below Lake Gregory, San Bernardino Mountains, from running clear water and gravelly sand; Mojave River, west fork, at Cedar Springs, at the mouth of Miller Canyon, clear water, gravelly sand, and algae; Dollar Lake, elevation 9300 feet, on the west flank of Mt. San Gorgonio, cold water and mud; and from a stream near Lake Arrowhead (at Cottage Grove Cabins), elevation about 5300 feet, clear water and coarse sand.

Allomyces species

Species of the genus *Allomyces* appear to be more drought-resistant than any other species of aquatic fungi. The author has been able to keep all isolates of this genus stored in a desiccated condition for long periods of time, up to 260 days in one case, and they can undoubtedly withstand longer periods of dryness. These cultures are readily renewable by inoculation with halved hempseed in water. *Allomyces* species have been isolated five times in the 733 samples of soil and water under study, four of the isolates being from desert localities.

Allomyces javanicus Kniep was isolated from Monument Valley in northern Arizona, from moist sand at the foot of a dry waterfall, near the Totem Pole, August 5, 1937; again from Snow Creek, at the base of Mt. San Jacinto, near Palm Springs, California, from moist, gravelly sand, at a depth of twelve inches, February 25, 1940. *A. cystogena* Emerson was obtained from the Gila River, near Yuma, Arizona, in very dry surface clay, March 16, 1940. *Allomyces* species have been isolated once in San Bernardino, from a deposit of mud and sand, after a heavy rain and run-off, in a street gutter near the junior college campus, January 1, 1941, and again from Calico Dry Lake, near Barstow in

hard-packed clayey mud, 4 inches deep (surface hard, compact, and dry), February 4, 1941.

Olpidiopsis species

Olpidiopsis Saprolegniae (Cornu) Fischer has been definitely identified once, then parasitizing *Saprolegnia* (a non-fruiting species), June 27, 1940, from a lily pond at the Loma Linda Sanitarium. *Olpidiopsis* has been procured on previous occasions, but accurate data of its frequency of appearance have not been kept.

SUMMARY

Collections of water and soil for the purpose of isolation of species of the Saprolegniaceae have been made in many localities between the eastern edge of the Rockies and the Pacific Ocean, in the United States, with the greatest emphasis up the southern California area.

At higher elevations and in the more northerly latitudes at lower elevations, and even near sea level, watermold species have been found at times to be scarce or non-existent, while at more southerly lower elevations species are usually very plentiful. Watermold species are commonly absent in the more arid regions. In regions of plenty, watermold species are isolated from both water and soil.

Of existing genera and species, *Saprolegnia ferax*, in two forms, has been taken most often, 75 and five times, respectively. *Saprolegnia diclina* and *S. delica* have been procured four and 17 times, respectively. *Saprolegnia* species, fruiting but unidentified, have been taken four times, and non-fruiting Saprolegnias, not classified elsewhere, 15 times. *Achlya caroliniana* has been locally plentiful in two small canyons, but found nowhere else to date. Unidentified fruiting Achlyas have been isolated eight times, while non-fruiting (unidentified) ones have been found nine times. *Aphanomyces*, non-fruiting, has been very abundant in many localities, having been taken 52 times. *Dictyuchus* species, non-fruiting, possibly in two forms, have been obtained 68 times, while a fruiting type of the genus has been found once. *Brevilegnia megasperma*, *Geolegnia inflata*, *G. septisporangia*, *Calyptralegnia unisperma* var. *litoralis*, and a non-fruiting *Pythiopsis* have been found a few times each.

Several new species of existing genera have been isolated: *Saprolegnia bernardensis*, so far confined to the higher elevations of the San Bernardino Mountains, two other very distinct species of *Saprolegnia*, both non-fruiting, *Achlya pacifica*, *A. pinnulata*, *A. diffusa*, *A. californica*, and *A. heteromorpha* (a heterothallic form), as well as *Aphanomyces balboensis*. Some previously established species have shown variation from their original description. .

From first meager results, endemism is suggested in the distribution of certain Saprolegniaceous species. (It should be recalled that certain members of the higher green plants are endemic to certain "island" areas in the same localities.) Work will be continued in order to verify or deny the possible existence of endemism.

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EXPLANATION OF PLATES

PLATE 1

Saprolegnia ferax, Type II, figs. 1–5

Saprolegnia species (non-fruiting #1), figs. 6–9

Figures 1–4. Oogonial habits. The elongated, filamentous to clavate oogonia, or combinations of oogonial types in series are characteristic of form II of *S. ferax*. In figure 3, note also a gemma. All figures × 335.

Figure 5. Gemmae. × 205.

Figures 6, 7. Branching hyphae and sporangial habits of a new, non-fruiting species of *Saprolegnia*. In figure 7, note the branched, immature sporangial initial. Both figures × 203.

Figures 8, 9. Types of gemmae. Gemmae in moniliform series, as in figure 8, are characteristic of the species. They are abundant after one week. Other oddly shaped gemmae, as in figure 9, are not uncommon. Both figures × 203.

PLATE 2

Saprolegnia species (non-fruiting #1), figs. 1–5

Saprolegnia species (non-fruiting #2), figs. 6–15

Figures 1–5. *Saprolegnia* species, non-fruiting #1. Types of sporangia. In this species, frequently spores are retained in the sporangia. In figure 2, note the large undischarged spore (or spore-like body) in the middle of the sporangium. Profusely branched sporangia, as in figure 5, are characteristic of water cultures three days old. Walls of such sporangia are thin and pliable, becoming bulgy as a result of internal pressure. All figures × 203.

Figures 6, 7. Primary sporangia of a second non-fruiting species of *Saprolegnia*. × 206.

Figures 8–13. Types of gemmae. The gemmae in this second new species of *Saprolegnia* show a uniqueness in their irregularity. However, in figures 8 and 9, the gemmae are quite regular, and in figures 10 and 11 they approach a condition similar to that existing in *Saprolegnia* species, non-fruiting #1. All figures × 75.

Figures 14, 15. Habit sketches, showing empty gemmae and also empty hyphae. Almost an entire mycelium may sporulate in a water culture before it is ten days old, as indicated in figure 14. Both figures × 75.

PLATE 3

Saprolegnia bernardensis

Figure 1. An intercalary oogonium. Such an oogonium may reach a length of more than one millimeter. × 337.
Figures 2–5. Types of oogonia and oospores. Antheridia may be absent or present, androgynous or diclinous. All figures × 337.
Figure 6. An oogonium resting on a nest of androgynous antheridial hyphae. × 337.
Figure 7. Proliferating oogonia. × 337.

PLATE 4

Achlya pacifica, figs. 1–7

Achlya pinnulata, fig. 8

Figure 1. Habit sketch of *Achlya pacifica*, showing the relationship of sporangia, oogonia, and diclinous antheridia. × 75.
Figure 2. Sporangial and branching habit. × 205.
Figures 3–7. Types of oogonia and oospores. All figures × 338.
Figure 8. Habit sketch of *Achlya pinnulata* to show the manner in which androgynous hyphae may arise at a distance, in this case more than one millimeter away. × 65.

PLATE 5

Achlya pinnulata

Figure 1. Habit sketch to show sporangial arrangement. Trichotomy is common. Positions of discharged spore masses are indicated by dotted lines. × 56.
Figure 2. A branched sporangium, which has retained some of its spores. Sporangia are frequently wrinkled, as indicated. × 102.
Figure 3. An empty sporangium showing many papillae, through which spores may have been discharged. × 102.
Figure 4. An unusual oogonium. Note its lobed nature, with each lobe bearing one or two oospores. × 338.
Figures 5, 6. Oogonial habits, showing proliferation, oospores, and diclinous antheridia. × 338.

PLATE 6

Achlya californica, figs. 1–3

Achlya diffusa, figs. 4–8

Figure 1. A thin-walled sporangium of *A. californica*. × 200.
Figure 2. An oogonial habit, showing proliferation. Note the nest of diclinous antheridia around the mature oogonium, as well as around the original oogonial initial. × 200.
Figure 3. A gemmal habit, showing characteristically short, lateral branches. Note also a portion of an empty sporangium. × 85.
Figures 4, 5. Proliferated oogonial-like bodies of *A. diffusa*. Both figures × 77.
Figures 6–8. Oogonial habits. Note variations in size and details of oospores. All figures × 337.

PLATE 7

Achlya pinnulata, fig. 1

Achlya californica, fig. 2

Achlya heteromorpha, figs. 3–7

Figure 1. A long, slender sporangium of *A. pinnulata*. × 67.
Figure 2. A typical sporangium of *A. californica*. × 200.
Figure 3. A branching habit of *A. heteromorpha*, male. In male strains of this species, sporangia are scarce, but gemmae are abundant. × 40.
Figure 4. Sporangia and gemmae of *A. heteromorpha*, female. In the female strains, sporangia are very plentiful. × 73.
Figures 5, 6. Characteristic gemmae of the female strain of *A. heteromorpha*. Both figures × 73.
Figure 7. Mature oogonia with oospores. Note abundance of antheridia. Obtained when male and female strains are produced. × 337.

PLATE 8

Aphanomyces balboensis, figs. 1–7

Dictyuchus species, figs. 8–11

Figure 1. Hyphae of *A. balboensis*, showing gemmae. × 215.
Figure 2. Sporangia habits. Note mass of spores that has floated up against the mature hypha at the left. × 215.
Figures 3–7. Oogonial habits. All figures × 585.
Figures 8–11. Oogonial habit of a fruiting species of *Dictyuchus*. Antheridia are always diclinous. All figures × 325.

PLATE 1

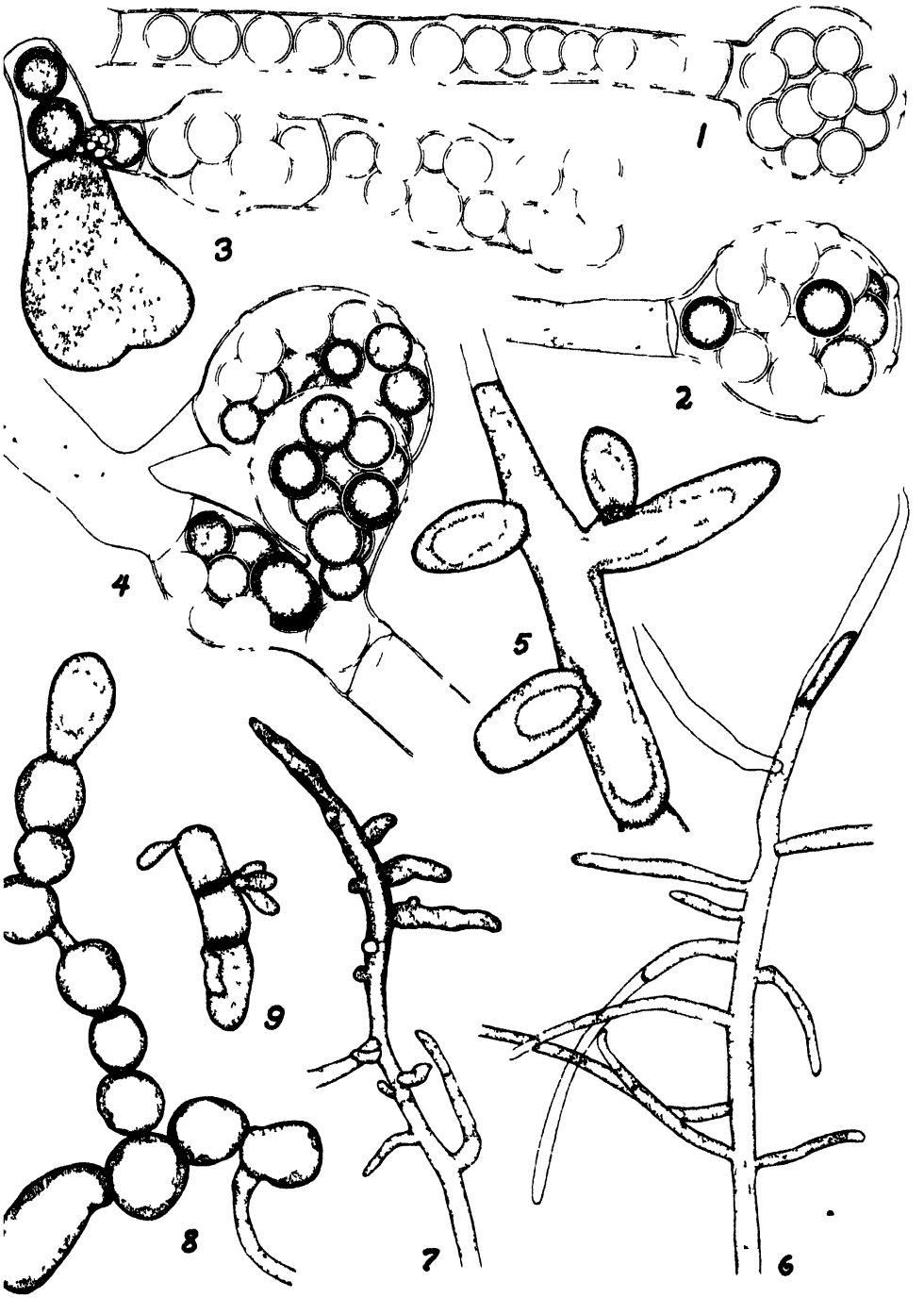


PLATE 2

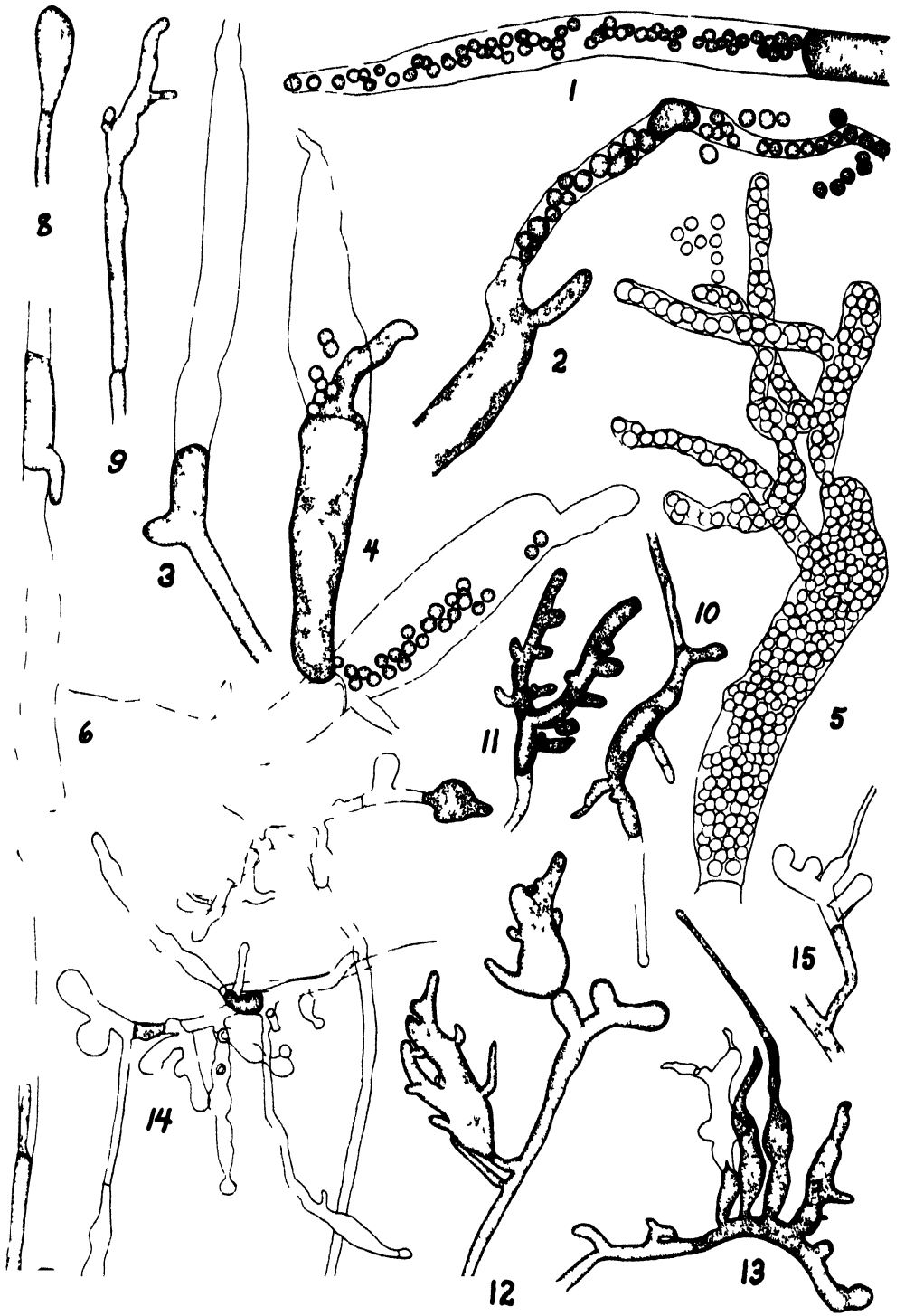


PLATE 3

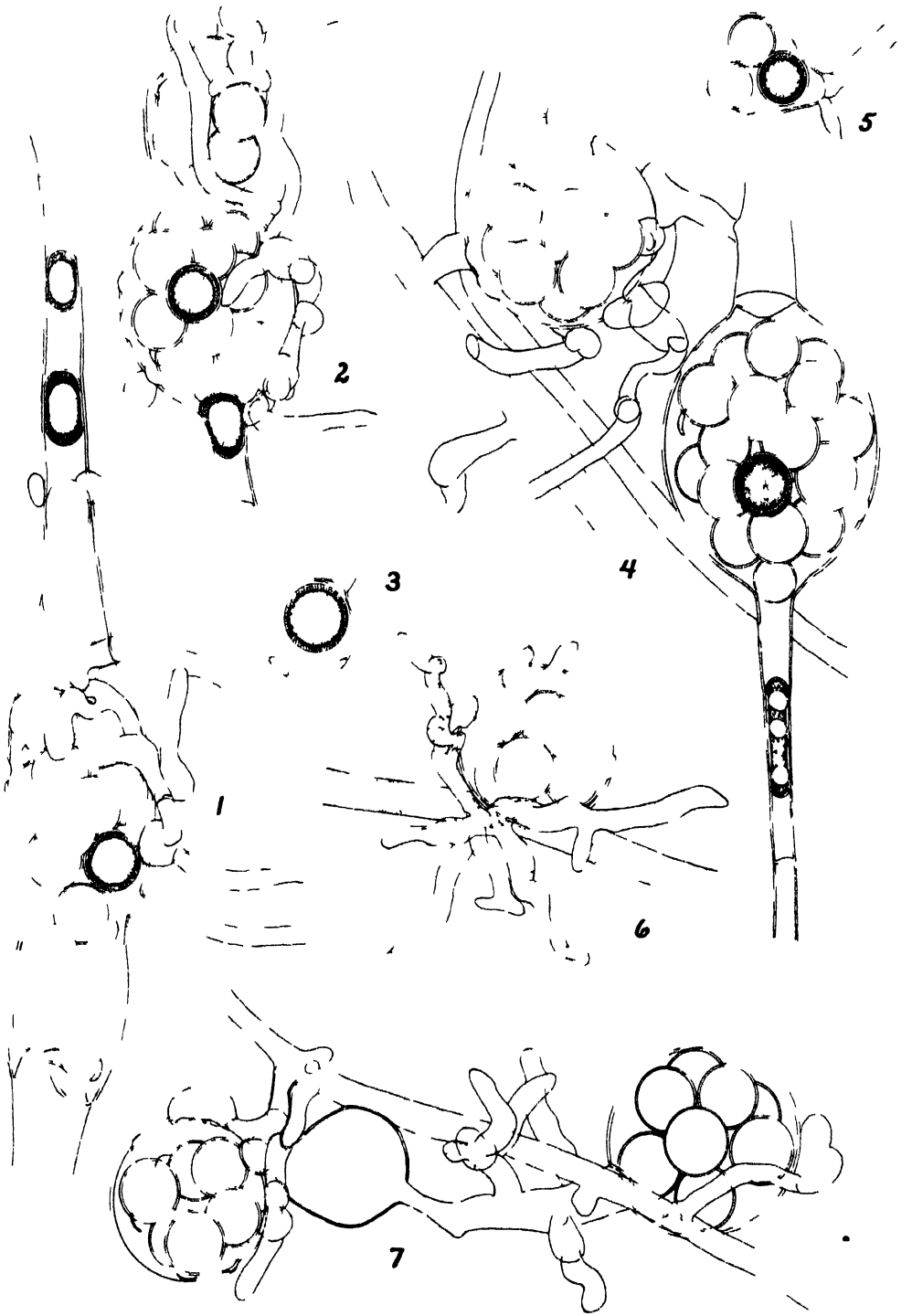
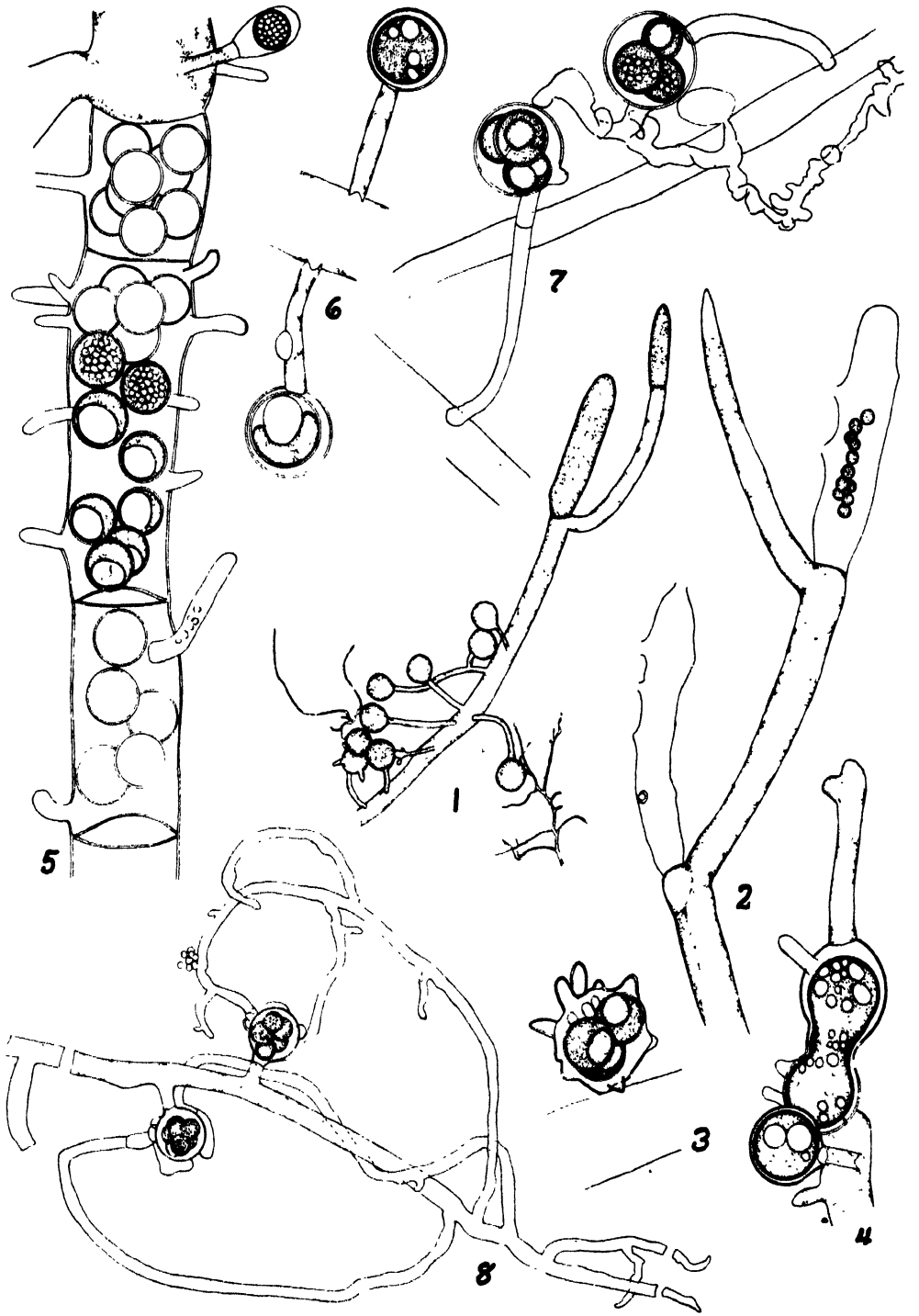


PLATE 4



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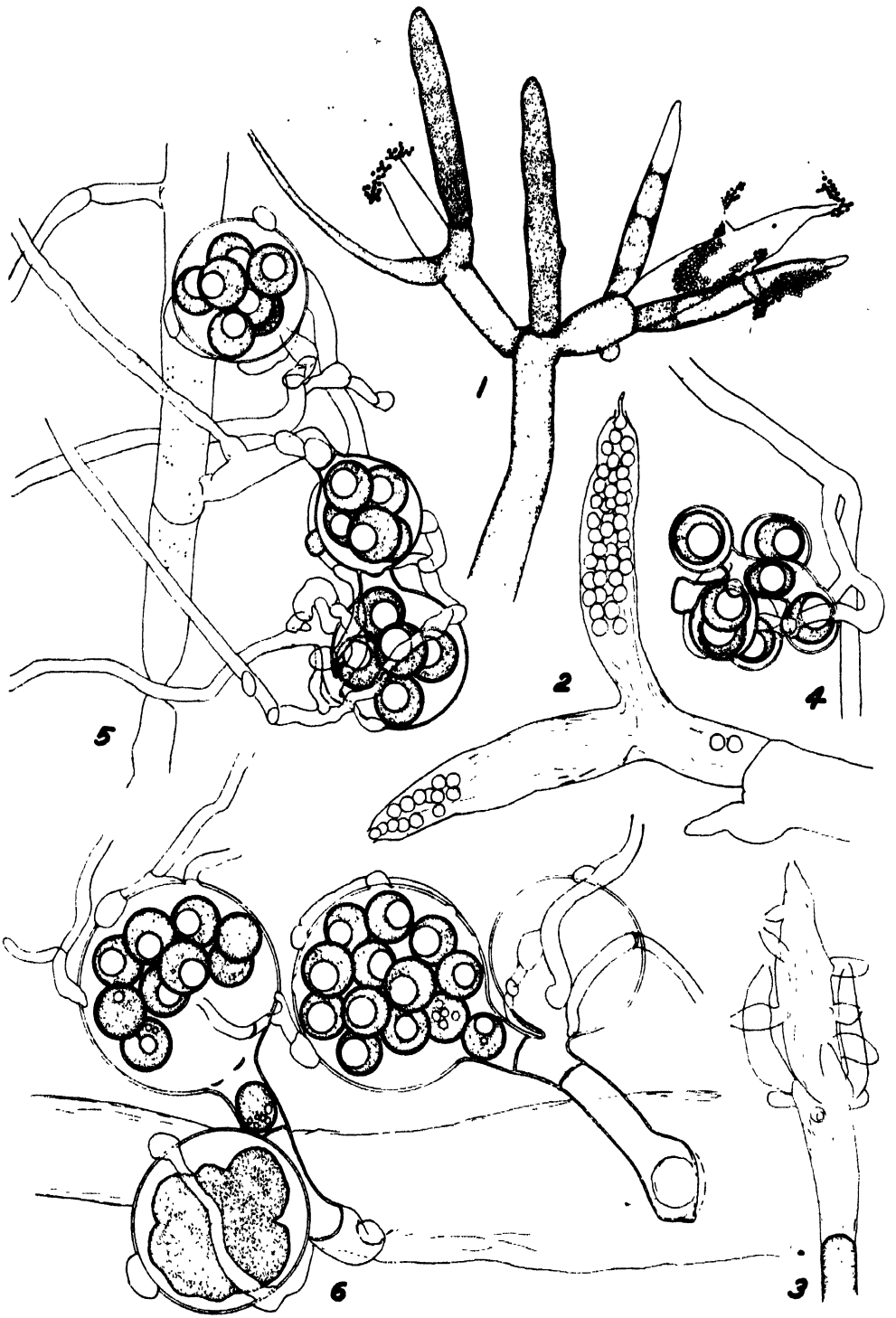

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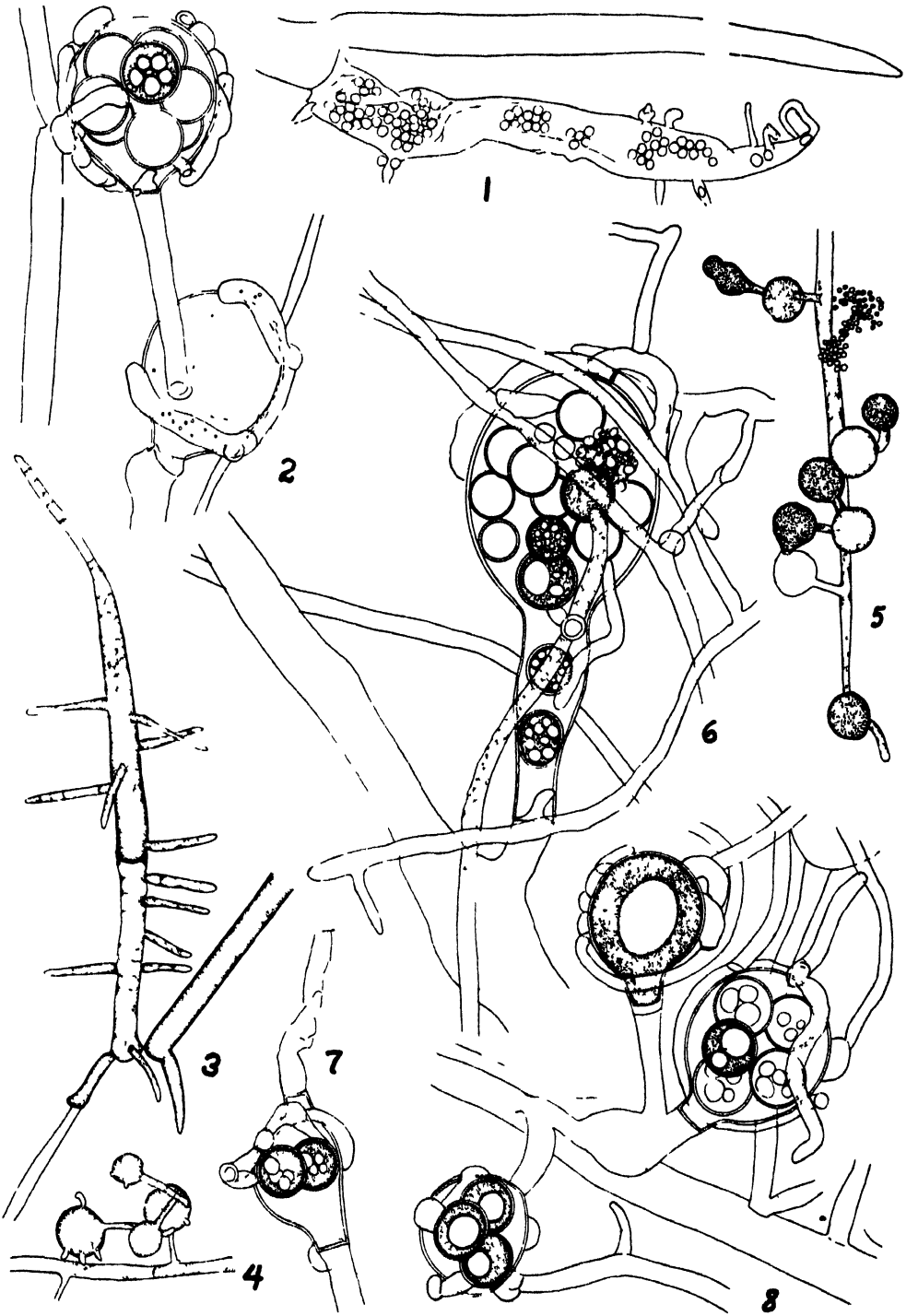

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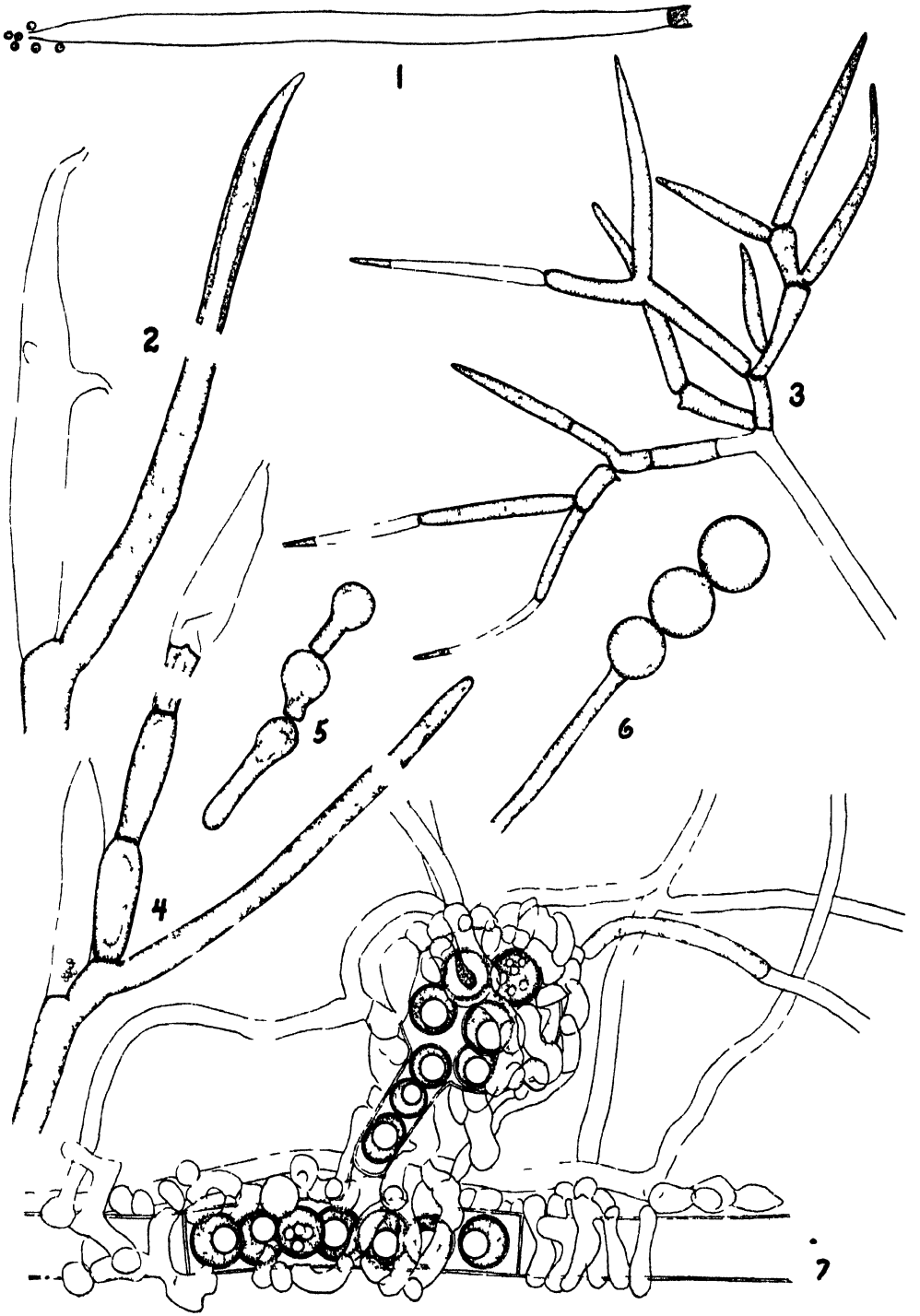

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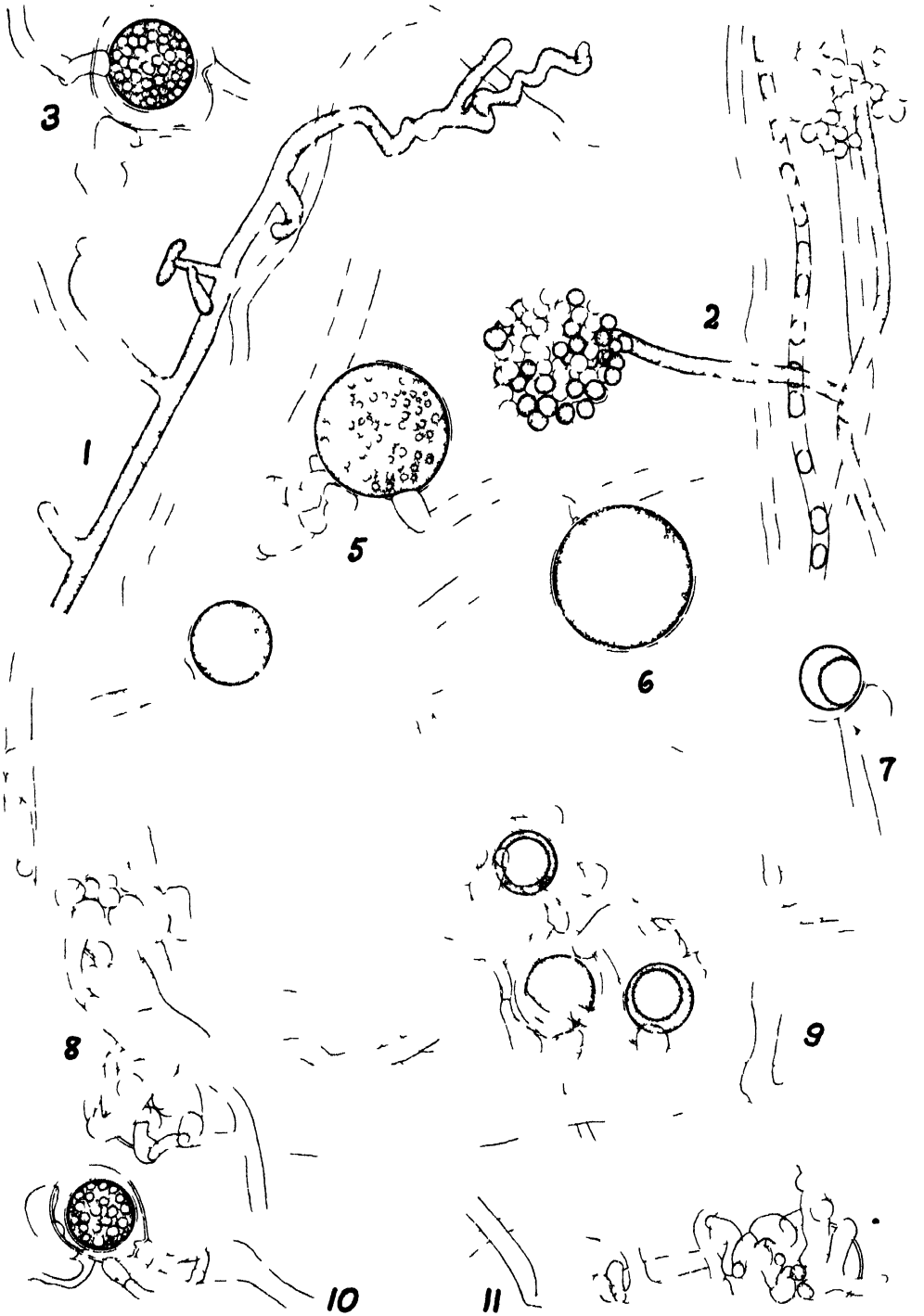

NUCLEAR PHENOMENA INVOLVED AT MEIOSIS IN COLEOSPORIUM HELIANTHI

By Lindsay S. Olive

Plates 9 and 10

INTRODUCTION

Coleosporium helianthi (Schw.) Arth. is a common rust in our area, with its uredinial and telial phases on a number of species of the genus *Helianthus* and its pycnia and aecia on the 2-leaved pines, *Pinus echinata* Mill. and *P. virginiana* Mill.

Earlier investigators who have studied teliospore germination and meiosis in the genus *Coleosporium* are Poirault and Raciborski (10) in 1895, on *Coleosporium euphrasiae* (Schumw.) Wint.; Sappin-Trouffy (11) in 1896, on *Coleosporium sonchi-arvensis* (Pers.) Lév.; Juel (6) in 1898, on *C. campanulae* (Pers.) Lév.; Holden and Harper (5) in 1902, on *C. sonchi-arvensis* (Pers.) Lév.; and Moreau (8) in 1914, on *C. senecionis* Pers., *C. sonchi-arvensis* (Pers.) Lév., and *C. melampyri* (Rebent.) Kleb. Holden and Harper and Moreau have gone into considerable detail on the study of nuclear fusion and meiosis in this genus.

The writer, in a detailed study of meiosis in *Coleosporium helianthi*, has found here certain important phenomena which apparently have been heretofore overlooked in this genus, or which might possibly occur only in certain members of the genus. The chief purpose of this paper is to present these new findings and to point out their implications in regard to the relationships of this type of rust to other groups of fungi.

MATERIALS AND METHODS

Diseased leaves of *Heliopsis scabra* Dunal. with abundant mature telia of *Coleosporium helianthi* were collected over the period October 6–16, 1940, at Chapel Hill, North Carolina. These telia appear in small, round, yellow spots on the under surface of the leaves and come out most abundantly toward the end of the growing season, from the middle of September to mid-October. They may germinate at maturity without a resting period, or, if the weather is not favorable for germination, the spores may retain their vitality for weeks.

The most satisfactory material for study was obtained by moistening the diseased leaves and then placing them, lower surface up, in a damp chamber at room temperature for about 5 hours. Some sporidia will have appeared by this time, but there will also be abundant meiotic divisions in progress. The material is transferred from the damp chamber to the killing and fixing fluids.

The killing and fixing agents used were formalin (10%)—acetic (5%)—alcohol (80%) and Flemming's weaker chromic-acetic-osmic acid solution. After having been washed and dehydrated in the usual manner, small squares of the leaves were imbedded in paraffin and sectioned 7–8 microns in thickness. Various stains were used, some proving much more satisfactory than others.

Gentian violet and safranin gave fairly good results, while Heidenhain's hematoxylin used in conjunction with light green was disappointing. Without the light green, the hematoxylin was the most satisfactory stain for bringing out nuclear details in all stages. The foregoing stains were all used after formalin-acetic-alcohol. The Feulgen staining technique was applied to material killed in Flemming's solution. It was used with excellent results in staining the dividing nuclei, but was rather ineffective in bringing out details of the nuclei in other phases.

INVESTIGATIONS

Examination of the sori with teliospores approaching maturity reveals the presence of a single large fusion nucleus in each spore (fig. 1). This nucleus is passing through a short resting period which is concluded as soon as the spore has matured in size, provided that conditions for germination are favorable. The fusion nucleus contains a single large nucleolus, which is stained intensely by hematoxylin, but not at all by the Feulgen stain. The chromatin is spread uniformly in a delicate reticulum. Many small vacuoles occur more or less evenly distributed throughout the spore protoplasm.

So far, the term "teliospore" has been used in describing the cells containing the fusion nuclei, but, for reasons which will be explained later, the more general term "probasidium" will be used hereafter.

Various stages in the transition of the nucleus from its resting condition into prophase of the first meiotic division and on up to spindle formation have been studied in detail. They are illustrated by figures 2–11. If some of the figures appear to be out of order, this is because those numbered from 6–11 have been arranged in order of certain important nucleolar developments, rather than with respect to chromatin condensation.

At the start of prophase the chromatin condenses from the network into what appears to be a long narrow and sinuous spireme (fig. 8). It is impossible to ascertain definitely whether there is one band of chromatin or several bands spiraling through the nucleus at this stage, but it seems more likely to be the former. Figures 2 and 11 show that what was once probably a single spiral band has now broken up into several shorter strands which have become more thickened. This is the leptonema stage, and the strands are the chromosomes, which, soon after their appearance, approach one another in pairs and fuse (fig. 3), the latter stage being known as **zygonema**. After fusion, these thickened chromosome bands begin to decrease conspicuously in length (figs. 4 and 5), resolving into the thickened little bodies which soon appear on spindles (figs. 5, 12–15).

The behavior of the nucleolus during prophase proved to be of particular interest. During the earlier part of prophase the nucleolus gives the appearance of being vesicular and not a completely homogeneous mass. Figure 6 shows the nucleolus in optical section. There is an outer darkly staining sphere and an inner region which assumes only a light purplish haze with Heidenhain's hematoxylin. As prophase progresses the nucleolus begins to show a double nature, as though it were splitting into two equal halves (figs. 7–11). Figure 8 shows the

nucleolus still circular in outline, but divided into two circular pieces, one above the other. This figure also shows the point at which each of these circles will be broken, so that they soon become more or less rod-like in structure (figs. 9–11). Figure 10 shows another interesting aspect. Each of the two rod-like pieces has a little connecting strand running from one end of it out to the nuclear membrane; while figure 11 shows the two little strands connecting the nucleolar pieces to two leptonemata in the nucleus. These leptonemata are similar in appearance and are probably homologous strands which will fuse during zygonema.

The nucleolar units are difficult to follow any further than this, for they take the hematoxylin stain just as the chromatin strands do. Furthermore, as the nucleolar units lose the purplish haze about them and approach the latter in size, they become indistinguishable from them. The nucleolar units, although they appear to be separate pieces, remain adjacent to one another as long as they can be detected during prophase. It is believed, however, that, by means of the little connecting strands, the two pieces are separated and carried by chromosomes during meiosis into the two new nuclei. Final proof of this is lacking at present. In reference to the figures showing these nucleolar developments, it should be noted that the nucleoli have been represented in heavy stippling, while the purplish haze which characterizes them is represented by a lighter stippling. This was done only to distinguish between the nucleoli and the chromatin of the nucleus, for the nucleoli stain just as the chromatin does with hematoxylin, and the purplish haze is not granular in appearance. Another important factor which should be brought out here is that the Feulgen method does not stain these nucleoli. This agrees with results obtained in higher plants.

Early stages in the formation of the spindle are usually somewhat obscure. At the time of the appearance of the first spindle fibers (fig. 5), the nuclear membrane may still be intact and may persist for a short while after anaphase is under way (fig. 12). On the other hand, the membrane may, in some cases, be broken down before the end of prophase (fig. 3). In any case the spindle should be considered of intranuclear origin, since the chromosomes, even when the nuclear membrane breaks down before spindle formation, continue to remain in the nuclear vacuole and not in the cytoplasm, and the spindle fibers appear exclusively within this vacuole (figs. 3, 5, and 12).

There is some indication that the chromosomes have already begun to split by the time the first spindle fibers appear. Figure 5 shows the chromosomes all drawn towards a central region in the nucleus and aligned in more or less the same direction, as though there were some magnetic field of attraction here. Though the first spindle fibers are appearing, most of the chromosomes that are shown appear already to have split without the aid of spindle fibers. The fibers, therefore, seem mainly to represent a field of attraction over which the chromosomes are drawn to the poles.

Figures 12–15 show typical anaphase spindles. They are generally characterized by having a distinct centrosome at each end (figs. 12 and 13), a varying number of spindle fibers, a number of small chromosomes, and, emanating from

the tips of the spindles, polar radiations. It should be pointed out here that the killing and fixing agents used, particularly formalin-acetic-alcohol, often cause an expansion of the parts involved at meiosis. This includes centrosomes, spindle fibers, chromosomes, and undoubtedly the polar radiations. This is of a decided advantage in the study of nuclear details which, under ordinary circumstances, might be inconspicuous and easily overlooked. This may explain why one is able to see distinct centrosomes in some figures, but not in others. For instance, figures 12 and 13 show considerably expanded chromosomes and spindle fibers, and the centrosomes are quite conspicuous; whereas, figures 14 and 15 shows less expanded chromosomes and fibers, and the centrosomes are not distinct.

At the tips of the spindles, the cytoplasm around the centrosomes is denser and much more finely granular than the surrounding protoplasm of the probasidium. Emanating from these denser areas are numerous polar radiations which appear early in anaphase and persist throughout telophase. In well-stained sections, they may often be seen extending from the tips of the spindles to the limits of the probasidial wall (fig. 15). These radiations will be discussed again presently, for they play an important rôle in later development of the basidium.

An attempt has been made to ascertain the number of chromosomes in this rust. As has been the case with most investigators of the rust fungi, the writer has found that the chromosomes here are too small to be counted with complete certainty of number. The clearest figures, however, indicate the presence of as many as 8 chromosomes for the haploid number. The anaphase spindle in figure 14 shows as many as 16 chromosomes passing to the poles.

Towards the latter part of anaphase, the spindle fibers usually draw out into two thickened strands (figs. 17 and 18). At the same time it is customary for the chromosomes to cluster into two masses at each pole (figs. 16–18). This is quite natural when one considers that the chromosomes are traveling to the poles along two paths and will therefore tend to congregate temporarily in two groups at each pole. This phenomenon is not peculiar to this rust, but has been found to occur in many rusts. Some investigators have mistaken these late anaphase figures as indicating the presence of 2 chromosomes.

By the end of anaphase and the beginning of telophase, an interesting change has begun to take place among the polar radiations. Some of them have begun to curve back towards the center of the cell, while the spindle fibers still persist as two strands between the dividing halves (figs. 19 and 20). It should be noted further that the dense cytoplasm at each end of the dividing nucleus has now become even more abundant. The polar radiations, as they curve around, frequently appear to intersect one another (figs. 20 and 21). It is at about this time that the spindle fibers disappear, while the cytoplasm between the two nuclei and bounded by the polar radiations curving backwards becomes less dense than the surrounding cytoplasm; that is, with the exception of a denser area of cytoplasm midway between the two nuclei in the region where the rays come together or intersect (fig. 21). A still later stage (fig. 22) shows that this

denser area across the center is resolving into a cross wall, and the rays from the nuclei are apparently contributing to its formation. In the various figures, rays may be seen emanating from the top, from the sides, and even from beneath the nuclei. There are evidently a number of these rays at the very top which never curve around, but continue to point towards the apex or base of the probasidium.

In early telophase the nuclei begin to reorganize. Little vacuoles appear within and at the sides of the chromatin mass (figs. 21, 22, and 22a), while the chromatin spreads out around these vacuoles. Figure 23 shows a reorganized nucleus following telophase. It is traveling towards the lower end of the cell. Its centrosome is attached to it and points in the direction in which the nucleus has been moving. The polar radiations and the dense cytoplasm around the centrosome still persist. Figure 24 shows the 2-celled probasidium at the end of the first meiotic division. This stage is not frequently found, as it is of very short duration. The nuclei never become very much expanded and go on almost immediately into the second meiotic division. Each nucleus has a lightly staining network of chromatin, while the nucleolus could not be distinguished from the numerous little clumps of chromatin on the network.

The second meiotic phase is initiated by division of the nucleus in each of the two cells of the probasidium. Figure 25 shows the two spindles in anaphase. Polar radiations have again appeared at the tips of each spindle. The spindles of the second division are smaller than those of the first, and the chromosomes are usually more difficult to distinguish. Figure 26 shows a dividing nucleus in a lower cell during late anaphase. Figure 27 shows the spindle in the lower cell in anaphase, while in the upper cell the nucleus has already divided, and a cross-wall is being laid down. It will also be noticed here that radiations from the nuclei run into this dense area midway between the two nuclei where the cross-wall is being evolved and are evidently contributing largely to its formation. In the lower cell, radiations may be seen extending over a considerable distance from the upper pole of the spindle to the cross-wall of the first division.

The result of the two meiotic divisions is a 4-celled basidium with a single haploid nucleus in each cell (fig. 28). Each nucleus has a delicate reticulum which stains only lightly with all of the staining techniques employed. Occasionally a small nucleolus may be seen (fig. 28a). The nucleoli are probably present in all of the nuclei, but they can not always be distinguished at this stage from the many little clumps of chromatin in the reticulum.

After the two meiotic divisions have been completed, there is a considerable enlargement of the basidium before it gives rise to the sterigmata, and the protoplasm becomes more conspicuously vacuolate (fig. 28). Each of the 4 cells then gives rise to a single long sterigma, which grows up to the surface of the gelatin enclosing the sorus. During this process, all of the protoplasm, including the nucleus, passes out into the sterigma. Figure 35 shows an unstained basidium taken from preserved material. It has produced 4 sterigmata, one of which was torn off. The protoplasm has just about finished passing from the cells into the sterigmata. In stained material the sterigmata show a very vacuolate nature

and contain nuclei that are in condensed form and stain quite darkly (figs. 29 and 30).

On reaching the surface of the gelatin, the sterigma, which has tapered off towards the tip, produces a little bulge just above the surface of the gelatin (fig. 30). This bulge gradually enlarges as the entire contents of the sterigma pass into it. This enlargement is the sporidium. A third nuclear division takes place in the sporidium shortly before all of the protoplasm from the sterigma has passed into it (figs. 31 and 32). The spindles of this division are quite small and indistinct. The result of the division is a binucleate sporidium. Shortly after this division the spore is shed, and one of the two nuclei degenerates. Figure 33 shows a sporidium which is germinating to produce a secondary sporidium. It contains two nuclei, one of which is degenerating. Secondary spore formation occurs when the sporidium falls into unfavorable environment, such as the gelatin of the sorus. Figure 36 shows a basidium with one sterigma (broken away) containing protoplasm, while the three others, following the production of sporidia, are empty. The wall of the sterigma (also of the sporidium) may be seen to be a continuation of the basidial wall, or the wall of what was formerly the probasidium. Figure 34 shows two sporidia taken from preserved material.

DISCUSSION

The so-called teliospore of *Coleosporium helianthi* has been referred to in this paper by the more general term "probasidium" because of its dissimilarity to the typical 2-celled and thick-walled teliospore of the higher rusts. Because it is 1-celled, thin-walled, and has only a short resting stage, or none at all, this probasidium is considered by such investigators as Sappin-Trouffy (12) and Linder (7) to be related to the *Auricularia* type of probasidium. As in the latter, both fusion and meiosis occur in the probasidium, and the basidia are similar, except for the lack of a clamp connection in the *Coleosporium* type.

In the process of cross-wall formation in the basidium of this rust, there are certain resemblances to ascospore delimitation as described by Harper (4) for such forms as *Phyllactinia corylea* and *Erysiphe cichoraccarum*, and by Dodge (3) for *Gelasinospora tetrasperma*. In *Coleosporium helianthi*, the astral rays cut out the cross-walls of the basidium; whereas, in the Ascomycetes mentioned, the astral rays cut out the circular ascospore wall. Linder (7), although he reports no such methods of cross-wall formation in the rusts, compares the rust basidium with a 4-spored ascus. He states that "the same four-celled structure is found in the rust basidium as is found within the extruded inner elastic wall of the Ascomycetes, but instead of being represented by free spores, they are represented by cells." The discovery of astral rays with wall-forming properties in the rusts should add further emphasis to Linder's views. In such a comparison, it should also be remembered that there is a third nuclear division which occurs in the sporidium before it is cut off from the sterigma. There is apparently no practical value in this division in *Coleosporium*, since one of the nuclei quickly degenerates. It suggests, however, a phylogenetic relationship to the third nuclear division in the ascus.

Colley (2) found polar radiations during meiosis in *Cronartium ribicola*. He reported that these radiations were long and prominent during anaphase and telophase, but concluded that they were probably due to cytoplasmic condensation and were not true astral rays.

The nucleolar history in *Coleosporium helianthi* is of particular interest. Sappin-Trouffy (11), E. W. Olive (9), Moreau (8), Savile (13), and others indicate that the nucleolus is thrown out into the cytoplasm to disintegrate during meiosis. The present writer, however, has found no such cases of nucleolar disintegration, and believes that the nucleolus is divided at meiosis and carried into the new nuclei. The splitting of the nucleolus during prophase into two similar halves which appear to be attached by small strands to chromosomes has, so far as we know, not been heretofore described for the rusts.

Allen (1) is one of the few who have indicated that the nucleolus in the rusts does not disintegrate during meiosis. She states: "It is perhaps a far cry from the nuclei of higher plants to those of rusts, but the regularity with which the nucleoles move together and fuse in the newly fused nucleus at once suggests that here, too, a nucleole occupies a definite locus in a chromosome. . . ."

Savile (13) does not believe that the so-called nucleolus of the rusts is a true nucleolus. He calls the dense sphere in the nucleus the endosphere, which he believes is mainly chromatin. He further states that its size and density depend upon whether the nucleus is in an expanded or unexpanded condition. His contention is that the true nucleolus lies within this endosphere. The present author, on the other hand, believes that the structure referred to as the nucleolus in the probasidial nucleus of *Coleosporium helianthi* is a true nucleolus, probably homologous with that of higher plants. The main reasons for this belief are: (1) fusion of the nucleoli as integral parts during nuclear fusion (see papers of Allen, Holden and Harper, and Moreau), (2) the fact that the nucleolus has a vesicular appearance and does not contain within it a body which could be interpreted as a nucleolus, (3) the fact that the probasidial nucleus is a fully expanded one with all of its chromatin, now in the outer region, staining with the Feulgen technique, but with the nucleolar sphere remaining unstained—a constant result obtained with the nuclei of higher plants, and (4) the splitting of the nucleolus during meiosis into two parts which apparently become attached to chromosomes.

The chromosome number for *Coleosporium helianthi*, as has already been stated, is believed to be approximately 8. Sappin-Trouffy reported the chromosome number as 2 for the majority of genera, including *Coleosporium*, that he investigated. This is apparently due, however, to a misinterpretation of the bilobed chromatin masses at the ends of late anaphase spindles, as already described in this paper. Holden and Harper (5) reported from 6 to 10 chromosomes for *Coleosporium sonchi-arvensis*. Moreau (8), like Sappin-Trouffy, reported 2 as the haploid number for the three species of *Coleosporium* investigated by her. Colley (2) suggests that Moreau's determination was probably inaccurate due to the use of insufficiently differentiated preparations. Colley reports that there are probably 8 chromosomes in *Cronartium ribicola*.

SUMMARY

1. In *Coleosporium helianthi*, the large fusion nucleus in the probasidium (teliospore) enters from a short resting period into prophase of the first meiotic division. The chromatin apparently condenses into a spireme.

2. Chromosomes become distinct and pair. The pairs condense into small thickened bodies which split as they arrange themselves on a conspicuous spindle. The spindle may appear before or after the nuclear membrane begins to disappear. The chromosome number is probably 8.

3. The nucleolus shows a double nature during prophase, and one half of it is believed to be carried over, attached to a chromosome, into each of the nuclei of division.

4. A centrosome with conspicuous polar radiations (astral rays) appears at each pole of the spindle in both meiotic divisions. The polar radiations curve back in late anaphase and in telophase to take part in cross-wall formation.

5. The first meiotic division results in a two-celled probasidium.

6. The second meiotic division ensues almost immediately. The two spindles in this division are smaller and less distinct than that of the first. The two divisions result in a four-celled basidium with a haploid nucleus in each cell.

7. Each basidial cell gives rise to a long sterigma, which, in turn, produces a sporidium at its tip.

8. A third nuclear division takes place in the sporidium before it is discharged. A binucleate sporidium results and one of the two nuclei degenerates soon after the spore is shed.

9. Sporidia may germinate to form secondary sporidia.

The author wishes to acknowledge the guidance and helpful criticism offered by Dr. W. C. Coker, under whose direction this work was performed.

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EXPLANATION OF PLATES

PLATE 9

(All figures × 1450)

Fig. 1. Probasidium with nucleus in resting stage.
2. Prophase with leptonemata.
3. Prophase with pairing strands.
4. Condensation of chromosomes, apparently after pairing.
5. Intra-nuclear spindle fibers appearing.
6. Vesicular nucleolus during prophase.
7. Prophase with nucleolus apparently beginning to split.
8. Prophase, showing spireme; nucleolus split circumferentially.
9. Prophase, showing two adjacent rod-shaped nucleolar units.
10. Prophase, showing connecting strands running from nucleolar units out to nuclear membrane.
11. Leptonema stage with nucleolar units connected to chromatin strands.
12–15. Early anaphase. Note spindle fibers, centrosomes, chromosomes, and polar radiations.
16, 17. Late anaphase with spindle fibers drawn into two threads and with chromosomes collecting in two groups at each pole.
18. Late anaphase with bilobed chromatin mass at each pole.
19. Telophase, showing astral rays bending back.
20. Telophase with astral rays bending back.
21. Telophase with dense streak of cytoplasm appearing across area where spindle fibers intersect. Note much lighter cytoplasm elsewhere between the dividing nuclei.
22. Late telophase, showing the first cross-wall appearing. Note astral rays running into cross-wall region.
22a. Reorganizing nucleus following the first division. Note vacuoles.

PLATE 10

(Figs. 23–33, × 1450; Figs. 34–36, × 475)

Fig. 23. Reorganized nucleus in a lower cell of the 2-celled probasidium. Centrosome points in the direction in which the nucleus has been traveling.
24. Two-celled probasidium.
25. Second meiotic division in anaphase.
26. Late anaphase in a lower cell.
27. Second meiotic division; anaphase in lower cell, telophase in upper.
28. Four-celled basidium.
28a. Basidial nucleus with a small nucleolus.
29. Upper end of sterigma before sporidial formation.
30. Sterigma with sporidium appearing at its tip.
31, 32. Nuclear division in the sporidium.
33. Binucleate sporidium giving rise to a secondary spore. Notice that one of the two nuclei is degenerating.
34. Sporidia taken from preserved material.
35. Basidium with three sterigmata, the apical one missing.
36. Basidium with four sterigmata, three empty and the lower, younger one disconnected.

PLATE 9

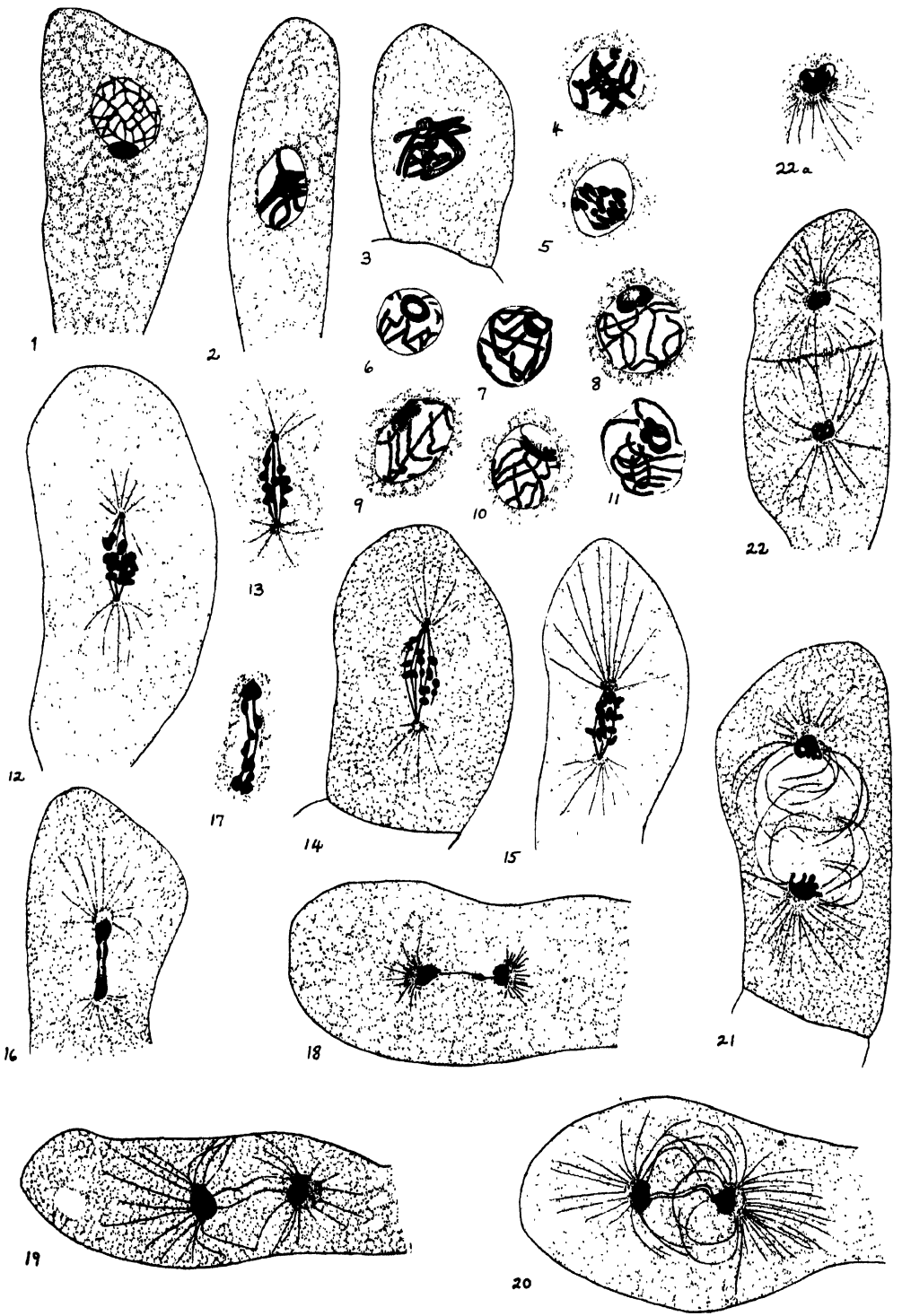
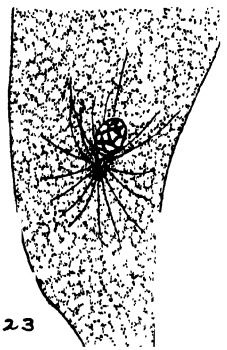


PLATE 10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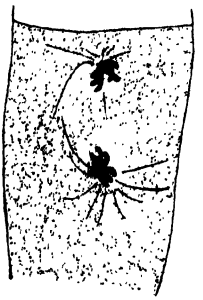
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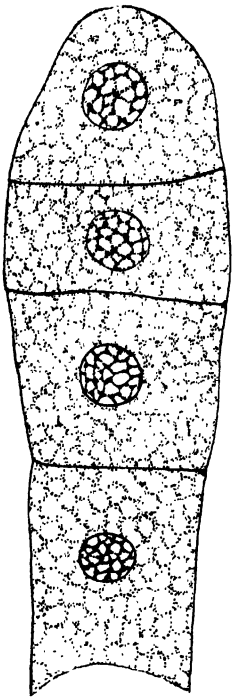
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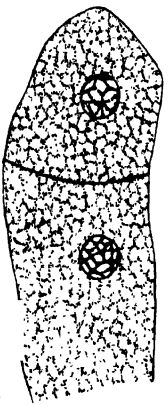
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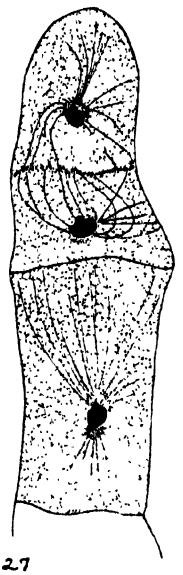
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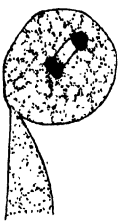
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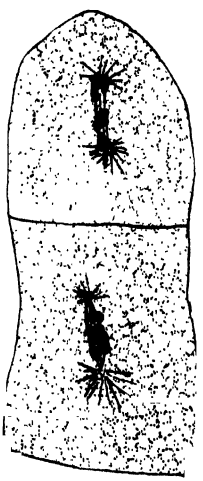
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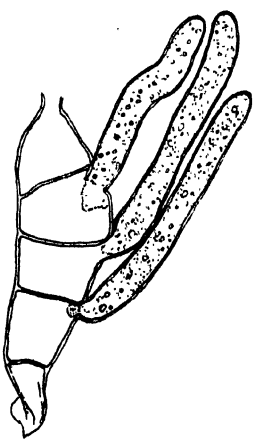
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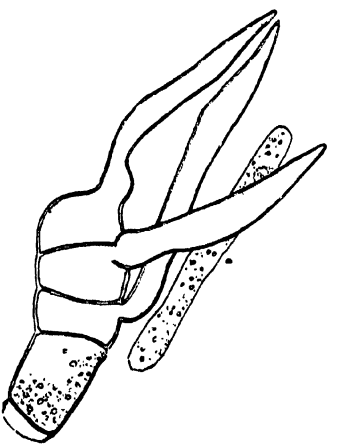
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THE EFFECT OF SOME SULFONAMIDE DRUGS UPON SEVERAL FREE-LIVING MICRO-ORGANISMS

By Frederick F. Ferguson, Jane R. Holmes, and Edward Lavor

The mode of action of the sulfonamide compounds upon living micro-organisms is still a controversial question. Considerable experimental evidence has appeared in the literature to support the theory that these drugs act directly upon the micro-organism, interfering in some manner with its normal metabolic activity. With few exceptions, these investigations have been concerned with bacterial cells. It seemed of interest to study the problem using free-living, aquatic micro-organisms. Such an approach may help to clarify some of the obscurity regarding the mechanism by which these chemicals act as therapeutic agents.

This paper represents the first of a series of studies on the effect of sulfonamide compounds upon certain free-living aquatic micro-organisms. It deals specifically with gross morphological changes and with histological changes observed in *Hydra*, rotifers, Protozoa, and other aquatic micro-organisms following exposure of these micro-organisms to certain sulfonamide drugs.

Since 1937 a vast literature has been amassed concerning the chemotherapeutic effects of sulfanilamide and related compounds upon viral, bacterial, protozoan and helminthic infections. A few workers have studied the toxic effects of these drugs upon the tissues of higher plants and animals. Grace (1938) showed that sulfanilamide acted as a growth-stimulatory substance for plants. In the same year Julius (1938), studying chick fibroblasts *in vitro*, found that although a 1:1000 dilution of sulfanilamide had no apparent effect upon the tissues, a 1:333 dilution was detrimental. Barnes (1940) has observed that a one per cent solution of sulfanilamide reduces the oxygen consumption of frog skin 40 per cent.

Van Dyke (1939) showed that when sulfonamide compounds were administered repeatedly to mice in food, sulfathiazole was more toxic than sulfapyridine at a high dose level, but at the therepeutic dose level no difference in toxicity could be demonstrated. In monkeys and growing rats receiving either drug over periods ranging from 14 to 51 days, sulfapyridine was the more toxic. Long (1940) noted that in mice the acute toxicity of sulfathiazole was one-third greater than that of sulfanilamide and about one-half that of sulfapyridine. Sulfathiazole was absorbed more readily than sulfapyridine.

MATERIALS AND METHODS

Organisms: The organisms employed in this study were brown *Hydra*, rotifers, *Stenostomum* sp., *Dero* sp., Crustacea and Protozoa including *Paramecium caudatum*, *Amoeba proteus*, *Halteria grandinella*, and various heterotrichs. Stock specimens were kept in aquaria and removed as needed.

Compounds: The sulfonamide drugs employed were sulfanilamide, sulfathiazole, sulfapyridine and sulfaguanadine. Solutions of the drugs were prepared in distilled water.

Methods: The animals were placed in varying concentrations of the compounds in open Stender dishes and were observed directly with the microscope. A series of tests were run for each concentration of the test sulfonamide on each respective group of organisms.

Specimens for histological examination were fixed in Beauchamp's fluid for 12 hours, embedded and sectioned. The sections were stained with Delafield's hematoxylin and eosin. Microscopic examinations of the sections were made employing an oil immersion objective.

TABLE 1

Effect of Sulfanilamide on Certain Free-living Micro-organisms

ORGANISM	CONCENTRATION OF SULFANILAMIDE	PERIOD OF EXPOSURE	OBSERVED EFFECTS
		hours	
Hydra	1.0%, 0.05%, 0.33%	3	Body swollen and glazed; tentacles broadened with fuzzy edges; marked epidermal disintegration.
Hydra	0.25%, 0.20%	3	Tentacular disintegration.
Hydra	0.16%, 0.14%, 0.12%	3	Slight tentacular disintegration; other tissues normal.
Hydra	0.11%, 0.10%	3	All tissues normal.
Hydra	0.25%, 0.20%	5	Same as 1% for 3 hours.
Hydra	1.0%	12	Dead.
Hydra	1.0%, 0.5%	24	Dead—endodermal disintegration.
Hydra	0.33%, 0.25%	24	Large, basal, translucent vesicles before death; cells discrete.
Hydra	0.20%	24	Dead—endodermal disintegration.
Hydra	0.16%, 0.14%, 0.12%	24	Still alive—retained ability to adhere to substratum.
Hydra	0.11%, 0.10%	24	Normal.
Rotifers	1.0%	3, 10, 24	Debility; majority dead in 10 hrs.; few resist 24 hours.
Stenostomum	1.0%	1, 10	Rapid epidermal disintegration; all dead in 10 hours.
Crustacea	1.0%	10	Approximately one-half still alive.
Dero (annelid)	1.0%	12	Disintegrated.
Heterotrichs	1.0%	120	Many still living.

RESULTS

The gross effects obtained with sulfanilamide, sulfathiazole and sulfapyridine are presented in Tables 1, 2, and 3. In general these compounds produced an extensive debility, marked body edema and epidermal epithelial sloughing accompanied by a loss of tentacles and decolorization in *Hydra*. The other forms studied varied in their sensitivity to the drugs and did not exhibit the extensive tissue damage observed in *Hydra*. The Protozoa were the least affected by exposure to the sulfonamides.

Sulfanilamide proved to be one of the least toxic of the compounds employed (Table 1). In one per cent aqueous solution (saturated) it was lethal for all

Hydra within 12 hours. In concentrations of one, 0.5 and 0.33 per cent extensive epidermal disintegration of the tentacles occurred within 3 hours. During this period the body appeared swollen and glazed and the tentacles became quite broad with fuzzy edges. Within 5 hours animals exposed to 0.25 and 0.20 per cent solutions exhibited these same phenomena. Tentacular disintegration was initiated within 3 hours when the organisms were immersed in 0.16, 0.14, and 0.12 per cent solutions of sulfanilamide, but the remainder of the tissues retained their normal appearance. Specimens exposed to concentrations of 0.11 and 0.10 per cent for 3 hours appeared to be unaffected.

When the period of exposure was increased to 24 hours, the effects produced in *Hydra* were more marked. Only those specimens immersed in concentrations of 0.20 per cent or less remained alive, whereas all others exhibited disintegration of the tentacles and epidermal epithelium followed by a similar disintegration of the endodermal epithelium. In 0.33 and 0.25 per cent solutions these animals showed large, basal, translucent vesicles prior to death, although the tissue cells remained discrete. Those exposed to 0.16, 0.14, and 0.12 per cent sulfanilamide, while losing some epidermis within 3 hours were still alive after 24 hours, as evidenced by their ability to adhere to the substratum. Those exposed to 0.11 per cent solution or less were apparently unaffected by the compound.

In one per cent concentration sulfanilamide was toxic to rotifers, causing debility within 3 hours and death of many within 10 hours. A 24-hour exposure killed all of them. *Stenostomum* underwent a rapid epidermal disintegration in this concentration of the drug and were dead within 10 hours. The Crustacea were relatively resistant, about half of them remaining alive after an exposure of 10 hours. The annelid, *Dero*, disintegrated within 12 hours when immersed in a one per cent solution. In the case of the heterotrichs, many were alive after 5 days' exposure to the saturated solution. In all of these forms normal functions were gradually regained, the degree of recovery being inversely proportional to the concentration of the drug. Protozoa persisted in an apparently normal state in concentrations of 0.33 per cent or less and *Amoeba* and *Halteria* increased in numbers in these lower concentrations.

The results obtained with sulfathiazole are given in Table 2. This drug had a more toxic and destructive action upon the tissues of *Hydra* than did sulfanilamide. When immersed in a one per cent solution, the organisms exhibited general debility, tentacular disintegration and body edema within 3 hours. Prior to their destruction, the tentacles displayed a central edema which resulted in a sixfold increase in their diameters. Within 12 hours the tentacles and epidermal epithelium had completely sloughed away, giving the animals an oblong appearance. Swollen tentacles, body edema and general debility were in evidence with all *Hydra* after 6 hours' exposure to all concentrations of the drug employed, although as the dilution increased, normal functions were regained. No impairment of function was observed in specimens exposed to concentrations of 0.11 and 0.10 per cent.

The effect of sulfathiazole upon Protozoa was less toxic than upon *Hydra*. At the end of 3 days' exposure to a one per cent solution, the organisms had lost their

motility, but the majority were viable and otherwise normal. After 7 days in this and decreasing concentrations of sulfathiazole, *Amoeba* were actively dividing and appeared to be unaffected by the drug.

Sulfapyridine proved to be the most toxic of the drugs studied (Table 3). When used on *Hydra* in concentrations of one, 0.5, and 0.33 per cent, it was instantly fatal; in lower concentrations the lethal effect was not evidenced until

TABLE 2

Effect of Sulfathiazole on Certain Free-living Micro-organisms

ORGANISM	CONCENTRATION OF SULFATHIAZOLE	PERIOD OF EXPOSURE	OBSERVED EFFECTS
		hours	
Hydra	1.0%	3	General debility; some tentacular disintegration; body edema.
Hydra	1.0%, 0.05%, 0.33%, 0.25%, 0.20%, 0.16%, 0.14%, 0.12%	6	General debility; some tentacular swelling; body edema.
Hydra	0.11%, 0.10%	6	Normal.
Hydra	1.0%	12	Body oblong shaped—complete disintegration of epidermal epithelium and tentacles.
Protozoa	1.0%	12	Loss of motility; few dead.
Protozoa	1.0%	160	Dividing normally.

TABLE 3

Effect of Sulfapyridine on Certain Free-living Micro-organisms

ORGANISM	CONCENTRATION OF SULFAPYRIDINE	PERIOD OF EXPOSURE	OBSERVED EFFECTS
Hydra	1.0%, 0.5%, 0.33%	Few seconds	Dead.
Hydra	0.25%, 0.20%, 0.16%, 0.12%, 0.11%, 0.10%	10 minutes	Dead.
Hydra	0.08%, 0.07%, 0.06%, 0.055%, 0.05%	12 hours	Dead; tissues very brittle.
Hydra	0.04%, 0.03%, 0.25%, 0.02%, 0.017%, 0.015%, 0.01%	12 hours	Normal.
Protozoa	0.25%	Few seconds	Dead.
Protozoa	0.125%, 0.06%	10 minutes	Markedly weakened.
Protozoa	0.05%	12 hours	Normal.

after 10 minutes. Sulfapyridine produced no edema, but there was a rapid sloughing of the tissues.

In order to observe the lethal process more adequately, each concentration of sulfapyridine was diluted with an equal volume of distilled water. By doing this, the toxic manifestations could be observed over a period of 12 hours (time of death for *Hydra*). Under these circumstances the brown pigment underwent

decolorization. At these higher dilutions there was no extensive sloughing, but the tissues became brittle, the tentacles breaking off easily, and the body wall becoming cracked and broken. This made the fixation of the tissue for histological study difficult.

Protozoa died promptly in concentrations of from 0.25 to 0.125 per cent of sulfapyridine and further dilution merely postponed the lethal effect proportionately. In a 0.063 per cent solution there was a disintegration of the organisms within 12 hours. In weaker solutions there was some decrease in motility, but otherwise the organisms appeared to be uninjured.

TABLE 4

Histopathological Changes in Hydra Following Exposure to Aqueous Solutions of Sulfonamide Drugs

COMPOUND	CONCENTRATION EMPLOYED	PERIOD OF EXPOSURE	HISTOPATHOLOGICAL FINDINGS
		hours	
Sulfanilamide	0 4% -0.8 %	24	Generalized necrosis.
Sulfathiazole	0.33%-0.6%	18	
Sulfapyridine	0.6% -0.12%	12	
Sulfanilamide	0 5%	24	Sloughing and disintegration of epidermal epithelium.
Sulfapyridine	0.3%	12	
Sulfapyridine	0.12%	12	Disintegration of intercellular substance. Morphological changes in cnidoblasts. Changes in mesoglea of tentacles.
Sulfapyridine	0.08%	12	
Sulfapyridine	0 33%	12	
Sulfapyridine	0.025%	12	Increased cnidoblasts transported into endoderm.
Sulfanilamide	0.8%	24	
Sulfanilamide	0.14%-0.2%	18	Increased number of interstitial cells.
Sulfapyridine	0.03%-0.125%	12	

Sulfaguanidine appeared to be less toxic than the other sulfonamide drugs studied. A concentration of 0.5 per cent was lethal for the *Hydra*, but in higher dilutions (0.25 to 0.1 per cent) a period of 36 hours was required before evidence of disintegration was discernible.

The results of the histological examination of 46 specimens of *Hydra* exposed to the sulfonamide compounds are summarized in Table 4. When specimens were exposed respectively to sulfanilamide (0.4–0.8 per cent) for 24 hours, sulfathiazole (0.33 per cent) for 18 hours, or sulfapyridine (0.06–0.125 per cent) for 12 hours, generalized necrosis occurred. Sloughing and distension of the cells of the epidermis occurred in specimens immersed in solution of sulfanilamide (0.5 per cent) for 24 hours and sulfapyridine (0.3 per cent) for 12 hours. Disintegration of the intercellular substance was observed only in a single specimen exposed

to 0.12 per cent sulfapyridine for 12 hours. Slight changes in the mesoglea of the tentacles were observed in one specimen after 12 hours exposure to 0.33 per cent solution of sulfapyridine. In another specimen immersion for 12 hours in 0.08 per cent solution of the same compound wrought morphological alterations in the cnidoblast cells. After a similar exposure to 0.025 per cent sulfapyridine or 24 hours exposure to 0.8 per cent sulfanilamide, there was an increase in the number of cnidoblasts being transported into the endoderm. The number of interstitial cells increased in 14 specimens exposed to sulfanilamide solutions (0.14–0.20 per cent) for 18 hours or to sulfapyridine solutions (0.03–0.125 per cent) for 12 hours.

DISCUSSION

These observations offer interesting opportunities for speculation. The marked edema which occurs after exposure to these compounds suggests that there is an alteration in cell membrane permeability which permits water to enter the tissues. The sloughing of the tentacles and epithelial tissue may be a mechanical rupture due to the plasmolytic effects of the edema. This is further suggested by the appearance of the translucent vesicles just prior to the death of *Hydra* exposed to sulfanilamide.

That such osmotic changes may be only secondary to a chemotoxic effect is possible, especially in the case of sulfapyridine. The decolorization of *Hydra* may indicate either a chemical union with the pigment or an alteration in oxidation-reduction potential of the tissue. Since edema was not marked when this drug was employed, and since the tissue was obviously altered in physical properties after exposure, a rapid chemical union might be postulated. If this resulted in a prompt loss of normal function, it would be impossible for the organism to adjust itself to any changes in membrane permeability.

The histological studies indicate that the necrosis of the tissues eventually becomes generalized. The reaction of the organisms to the drugs exhibits a characteristic pattern. The animal exhibits coiling and writhing when first immersed in the sulfonamide solution. Following this the body becomes glazed and bloated and the tentacles begin to show evidence of the destruction of the epidermis. The epidermal epithelium subsequently peels off, forming a mass of cells at the base of the animal. Concomitantly the tentacles are reduced to mere nubs and the animal assumes an ovoid shape just prior to disintegration.

The histopathological changes induced by the sulfonamide compounds appear to be defensive in character. This is evidenced by the increase in numbers of interstitial cells which possess potential regenerative powers.

SUMMARY

1. Sulfanilamide, sulfathiazole, and sulfapyridine in aqueous solution produce a marked effect upon certain free-living aquatic micro-organisms, especially *Hydra*. Exposure to these drugs results in a rapid disintegration of the tissues accompanied by body edema, decolorization and increased brittleness of the tissues.

2. Protozoa appear to be more resistant to these compounds than the metazoa.

3. The order of toxicity of these compounds is: sulfapyridine (most toxic), sulfathiazole, sulfanilamide, and sulfaguanidine (least toxic).

4. Sulfaguanidine uniformly decolorizes *Hydra* without obviously interfering with its normal functions.

5. Histopathological studies were made on specimens of *Hydra* immersed in various concentrations of sulfonamide compounds in aqueous solution for varying periods of time. In these specimens there was a general necrosis of all tissues accompanied by a sloughing and distension of the cells of the epidermal epithelium. There was a modification of the tentacular mesoglea. The cnidoblasts in the endoderm were increased in number and exhibited an abnormal morphology. The numbers of interstitial cells were noticeably increased.

The authors wish to express their gratitude to Dr. Leslie A. Sandholzer for his interest and technical advice and to Dr. W. A. Kepner for the examination of the histological material.

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QUERCUS MACROCARPA IN ALABAMA

By Roland M. Harper

Plate 11 and One Text Figure

Quercus macrocarpa, described by André Michaux (Hist. Chen. Am., pl. 2) in 1801, and commonly known as bur oak or mossy-cup oak, is a characteristic tree of the upper Mississippi valley, where it grows typically in prairie groves. In the original description it was said to grow in upland clayey or calcareous soils everywhere west of the Alleghanies, particularly in Kentucky, Tennessee, and Illinois. It is now said by various authorities to range eastward to Nova Scotia, northward to Manitoba, westward to northeastern Wyoming, and southward to central Texas. The southeastern edge of its range is ill-defined, but it seems to be very rare east of the Alleghanies, and some of the reported localities in that direction seem very questionable, and may be based on cultivated trees, or specimens wrongly identified or inaccurately labeled in herbaria.

Chapman in 1860 gave its distribution as "North Carolina and northward"; but it is not mentioned in Pinchot and Ashe's excellent monograph on the trees of North Carolina, published in the spring of 1898, and Coker and Totten, in their little book, "Trees of the Southeastern States" (1934), express the opinion that the North Carolina specimens referred to *Q. macrocarpa* are more likely *Q. bicolor* Willd. (*Q. platanoides* (Lam.) Sudw.).

Small, in his Flora (1903) and Manual (1933), extends its range southeastward to Georgia; but I have been in every county in Georgia, and the only *Quercus macrocarpa* I have ever seen in that state is a large tree in Athens, in a ravine on or near the site of a botanical garden maintained by one of the university professors about the middle of the 19th century. It bears very few acorns, and the pickaninnies living near by usually grab them for souvenirs or playthings as fast as they fall.

Gattinger, in his Flora of Tennessee (1901), says of it, "O.S.", meaning "over the state," which is certainly an exaggeration. I have been in most of the counties of Tennessee, and cannot recall ever seeing *Quercus macrocarpa* there.[1] It is not mentioned in Mohr's Plant Life of Alabama (1901), or Lowe's Flora of Mississippi (1921).

In October, 1927, Mr. Haygood Paterson, a florist of Montgomery (now state commissioner of agriculture), took me to a medium-sized tree of this species at

[1] One botanist, who has collected extensively in Tennessee, writes me that he has never seen *Quercus macrocarpa* growing in that state, but has a good specimen from Nashville. Another, who has lived in Nashville a good many years, says it is "exceedingly common" in Tennessee, and he thinks he has seen it as far south as Columbia and Mount Pleasant, if not near Tuscumbia, Ala.

Evidently botanists (the writer perhaps included) sometimes pass this tree without recognizing it. More information about its occurrence in Virginia, West Virginia, Maryland, and east of the Berkshires in New England, would be interesting.

the edge of a field near Snowdoun, Montgomery County, in the heart of the black prairie belt. Its habitat was similar enough to that of trees I had previously seen in the Mississippi valley, but as there was only one tree in sight, and that at the edge of a field, and 200 miles or more from any previously known station for the species, its indigeneity seemed doubtful. I reported it the following year in my publication on the woody plants of Alabama (Geol. Surv. Ala., Monog. 9, p. 114), but with considerable skepticism.

A few years later Mr. T. L. ("Tree Lover") Head, assistant superintendent of education of Montgomery County, became interested in the same tree, and in the fall of 1933 and later gathered many acorns from it, and had them planted by himself and friends in various places in and around Montgomery. One of the resulting seedlings, in front of his home, reached a height of more than twelve feet in eight years, but the others some of them younger—are smaller.

Mr. Head used to visit the tree every fall, but in the fall of 1939 learned that it had been cut down a few months before by some ignorant farm hand. However, about that time he discovered a larger tree of the same species in woods about a quarter of a mile away. He conducted me to that one on March 24, 1941, and I took some pictures of it, and some notes on the associated trees. It was about three feet in diameter and fifty feet tall, and grew in a pastured grove of large deciduous trees, mostly oaks, such as can be seen in many places in the blue-grass region of Kentucky, the Nashville basin, the black belt of Alabama, and other calcareous regions.

On account of many years of grazing by cattle in such places, the herbaceous vegetation of the grove is sparse and somewhat weedy, there are practically no shrubs, and there is little opportunity for tree seedlings to establish themselves. But the trees are all or nearly all native, and the bur oak in question must be about 100 years old, and it does not seem likely that any one would have planted it as long ago as that, in just the sort of place where it should grow naturally.

However, one or two specimens, far removed from other known ones of the same species, are a pretty slim basis for a locality record. So I suggested to Mr. Head that if *Quercus macrocarpa* is really native there, there should be other specimens somewhere in the vicinity, and urged him to search for them. In Wisconsin or Iowa, where there is no other oak likely to be confused with it, it can be recognized about as far away as any other tree; but in Alabama it could easily be mistaken for either *Q. stellata* or *Q. Michauxii*, until one came close enough to get a good look at its leaves or acorns.

Mr. Head, with a few interested friends, followed my suggestion, and in a few months reported finding a few more bur oaks in another grove about a mile north of the first one. He took me there on Nov. 28, 1941, and soon found some additional trees in the same grove, making about ten in all. This seems to establish it pretty well as a member of the indigenous flora of Alabama.

Its principal arborescent associates in the two groves, in approximate order of abundance, are about as follows: —*Quercus Schneckii*, *Q. Durandii*, *Celtis mississippiensis*, *Ulmus americana*, *U. alata*, *U. fulva*, *Quercus Muhlenbergii*, *Q. lyrata*, *Hicoria ovata*, *H. myristicaeformis*, and *Tilia* sp. There are also in the second

grove a few small trees, *Morus rubra*, *Crataegus spathulata*, *Cercis canadensis* and *Ilex decidua*, and a few shrubs and vines, *Rhus radicans*, *Bignonia crucigera*, *Smilax* sp., and *Sabal minor* (if that can be called a shrub). *Phoradendron flavescens* is parasitic on the *Celtis*, if not on other trees.

This widely scattered distribution of *Quercus macrocarpa* recalls that of a shrubby species of the same genus, *Q. prinoides* Willd., which I found in dry woods in the same county in 1927, but nowhere else in Alabama. Its range is chiefly northeastward, but I have never found it even in Georgia.

Figure 1 *Quercus macrocarpa* in leafless condition, in grove about a mile north of Snowdoun, Ala., March 24, 1941.

More similar to *Quercus macrocarpa* in distribution is the pecan, *Hicoria Pecan* (Marsh.) Britton, native chiefly in the Mississippi valley, but widely cultivated, and developed into several improved varieties, in the southeastern states. Late in the 19th century Dr. Mohr was shown a few specimens in the black belt of Alabama which were native according to local traditions;[2] and I investigated

[2] Charles Mohr, Garden & Forest **6**: 372-373, 1897, Contr. U. S. Nat. Herb. **6**: 100-101, 461-462 1901.

some of them with the state forester in 1924, and reported on them in the work previously cited (Monog. 9, pp. 89, 90).

The description of *Quercus macrocarpa* in Robinson and Fernald's Manual (the so-called 7th edition of Gray's), 1908, notes that the acorn cups are extremely variable. That is true enough, but it does not bring out the fact that great variations can be found on the same tree or at least in the same grove. In the grove visited last fall there were hundreds if not thousands of acorns on the ground, and I was impressed then with their variability, which I had not noticed before. If the extremes were confined to different regions they could easily be made the basis of subspecies if not species; but occurring together as they do, they probably have little significance.

At my suggestion Mr. Head and his friends collected a series showing some of the variations, and had them photographed, with some of the leaves; and one of the photographs is reproduced herewith. Incidentally, the illustration of this species in Sargent's Manual of Trees is either poorly drawn, or represents an extreme with thin and sparingly fringed cups; though it is indeed there indicated that the acorns are larger and the cups more copiously fringed in the South than in the North. That in Hough's Handbook of Trees (1907), made from a photograph of a Kentucky specimen, is much better, but still does not show quite as copious a fringe and wide cups as some of the Alabama acorns.

It is a matter of passing interest that Snowdoun is on the old Federal Road, which was previously an important Indian trail, that William Bartram is supposed to have followed on his way from Georgia to Mobile just before the Revolution. So apparently he just missed discovering *Quercus macrocarpa*, at the extreme southeastern edge of its range, a few years before Michaux found it farther north.

Supplementary note. Since the foregoing paragraphs were written another interesting characteristic of *Quercus macrocarpa* has come to light. Some of the acorns gathered last November were planted in boxes in my office early in December, and watered nearly every day, and in the spring about half of them germinated. The primary leaves, three or four in number, three or four inches long and about half as wide, aggregated at the top of a stem about six inches tall, with a few small scale-like bracts below, were not lobed like those on mature trees, but sinuate-toothed, suggesting *Quercus Michauxii* (*Q. Prinus*) rather than *Q. macrocarpa*. It would be a comparatively easy matter to study the seedling stages of other oaks in this way, and if that were done some new light might be thrown on their relationships.

UNIVERSITY, ALA.

PLATE 11

LEAVES AND ACORNS OF QUERCUS MACROCARPA FROM GROVE ABOUT TWO MILES NORTH OF SNOWDOUN, ALA., COLLECTED BY T. L HEAD AND OTHERS, NOV 28, 1941, AND PHOTOGRAPHED SOON AFTERWARD

About $\frac{2}{3}$ nat. size

PLATE 11

MASSOSPORA TIPULAE SP. NOV. AND TIPULA TRIPLEX COLEI ALEXANDER SUBSP. NOV.

By J. P. Porter[1]

One Text Figure

While collecting near Knoxville, Tennessee, on April 21, 1938, Dr. A. C. Cole, Jr., and Mr. Carl Huffaker of the Department of Entomology, University of Tennessee, found a new sub-species of crane fly (*Tipula triplex colei* Alexander), parasitized by a species of *Massospora*. Comparison of the material collected with other species of *Massospora* would seem to warrant specific rank, and the name *Massospora tipulae* sp. nov. is proposed.

Four species of *Massospora* have been described previously:

Massospora cicadina Peck (7), on which the genus was founded, develops within the body of the seventeen-year locust, *Magicicada septemdecim* (Linn.).

Massospora Staritzii Bresadola (1) was found in larvae of unknown insects. Speare (8) reduces *M. Staritzii* to a synonym of *Sorosporella uvella* (Krass.) Gd.

Massospora Richteri Bresadola and Staritz (2) was described from the bodies of unidentified flies. Bubak (4) subsequently reduces this species to a synonym of *Entomophthora Richteri* (Bresadola and Staritz) and *Entomophthora Lauxaniae* Bubak. From the imperfect descriptions, it seems that the validity of *Massospora Richteri* Bresadola and Staritz should be tentatively regarded until study of the type material is made.

Massospora Cleoni Wize (10) was described from the dipteron *Cleonus punctiventris* Germ.

Following is a tabular comparison of species of *Massospora*.

	M. cicadina	*M. Richteri*	*M. Cleoni*	*M. tipulae*
Resting spores size	38–50μ	53μ	25–30μ	41–49μ
Color in mass	russet	reddish	orange red	black
Surface markings	reticulations	irregular large warts	spines	irregular small warts
Conidia: size	16–21μ	39μ	(not reported)	(not found)
Conidia: color in mass	flesh colored to white	reddish		
Host	*Magicicada septemdecim and Magicicada tredecim*	*Lauxania aenae*	*Cleonus punctiventris*	*Tipula triplex colei*

Massospora tipulae sp. nov.

Sporae quiescentes endogenae, crassis parietibus, verrucis irregularibus, rotundae aut paenae, 41–49μ, insecti (*Tipula triplex colei* Alexander) abdomen implent; colore (cream buff), nigris verrucis: conglobatae in mole videntur nigrae. Pars cellae sporogenitivae tenuibus parietibus (it est corpus hyphale) ad sporam haerens tenuem planum parietem in quo loco affixa erat. Corpora

[1] Contributions from the Botanical Laboratory, The University of Tennessee, n. ser. No. 56.

hyphalia sunt sine colore, forma irregulare, plerumque sunt unicellaria quae facile dirumprentur, mycelium praeter corpora hyphalia non visum est. Conidia non reperta sunt.

Exemplae e Knoxville, Tennessee, April 21, 1938, A. C. Cole, Jr., and Carl Huffaker, in Universitatis Tennessensis herbario numerus 4400.

Resting spores endogenous, heavy-walled, irregularly warted, spherical to subspherical, 41–49μ, filling the abdomen of the insect (*Tipula triplex colei* Alexander); cream buff (Ridgway) with black warts, appearing black in mass. Part of the thin-walled sporogenous cell (hyphal body) remaining attached to

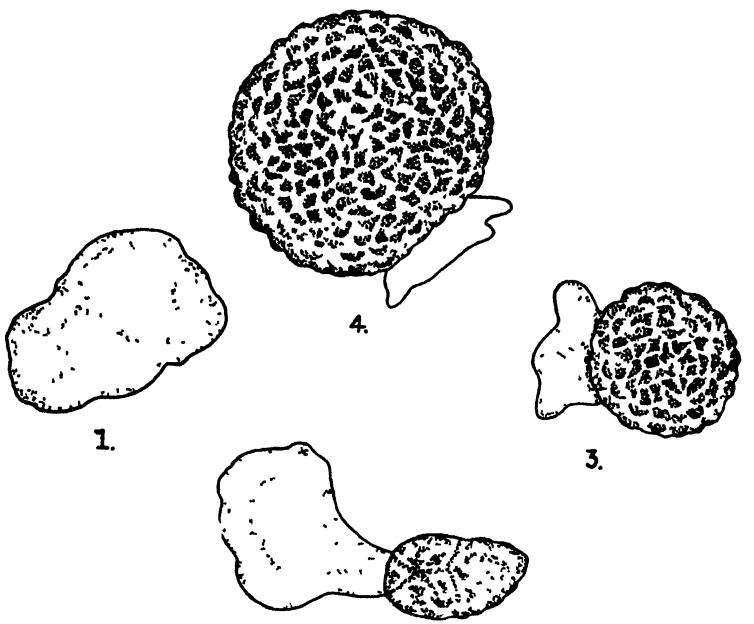

Fig. I. × 750.

1. Hyphal body.
2. Hyphal body with resting spore beginning to form.
3. Further stage in the formation of a resting spore; the hyphal body still containing cytoplasm.
4. Mature resting spore with the remains of the hyphal body attached.

Drawings by Alice Caton.

the spore forms a cup or breaks loose completely, leaving a thin, smooth wall at the place of attachment. Hyphal bodies colorless, irregularly shaped, usually one-celled, breaking apart easily. Mycelium other than hyphal bodies not observed. Conidia not found.

Type specimen from Knoxville, Tennessee, April 21, 1938, A. C. Cole, Jr., and Carl Huffaker, in Herbarium of the University of Tennessee, No. 4400.

Cytological work on *Massospora cicadina* Peck (6) indicated that spores are formed at night. In an attempt to observe sporogenesis in *M. tipulae*, collections were made at various hours of the night from 7:30 p.m. to 6:30 a.m. The speci-

mens, including obviously diseased to apparently healthy individuals, were placed immediately in a killing solution. Mature spores only were found in collections made after 9:00 p.m. and very few immature spores were present in the collections taken earlier in the evening.

An unsuccessful attempt was made to germinate the spores in distilled water and on the living insects.

In the springs of 1939 and 1940 the host was not found in this locality. Hence, no further collections of the fungus were made. In the spring of 1941 the fungus was found in the same locality by Dr. Cole on another species of *Tipula*.

NEW HOST FOR *Massospora cicadina*

On June 14, 1940, in Union County, Tennessee, near Big Ridge Park, one specimen of *Magicicada tredecim* was found parasitized by *Massospora cicadina* Peck in the conidial stage. Though the insects were abundant in the region, no other diseased specimens were found.

Collected about 4:00 p.m.; the insect was dead about 6:30 p.m. Among the mass of conidia a few hyphal bodies were found from which spores were developing.

Tipula (Lunatipula) triplex colei Alexander subsp. nov.

Characters as in typical *triplex* Walker (northeastern North America), differing chiefly in certain details of structure of the male hypopygium. Ninth tergite with the submedian points only slightly produced into low obtuse or subtriangular lobes, separated by a narrow, V-shaped notch. The degree of production of these submedian lobes is the slightest of any of the species or races so far discovered in the *triplex* group. Eighth sternite with the submedian lobes relatively broad, narrowed outwardly, obtusely rounded at their apices. In typical *triplex* the tergite has the submedian lobes produced into long acute spines that are contiguous at the midline, or separated from one another only by a very deep capillary incision. Eighth sternite with the submedian lobes narrow, almost parallel-sided for the entire length.

Holotype, ♂, Knoxville, Tennessee, at University Farm, May, 1938 (A. C. Cole).

Type of this subspecies is retained in my collection of crane-flies (C. P. Alexander).

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TWO NEW TURBELLARIA (ALLOEOCOELA) FROM BEAUFORT, NORTH CAROLINA, BELONGING TO THE NEW GENUS PREGERMARIUM

By M. A. Stirewalt, F. F. Ferguson, and Wm. A. Kepner

Plate 12 and One Text Figure

These new alloeocoeles were taken from marine algae below water level at Beaufort, North Carolina, during the summer of 1941.

We acknowledge the help given us by Dr. H. F. Prytherch in placing the facilities of his laboratory at our disposal and the financial aid given us by the Virginia Academy of Science and the Research Committee of the University of Virginia.

PREGERMARIUM new genus

Cylindrostominae with a single ovary lying anterior to the cephalic ganglia; paired, lateral vitellaria; paired, compact testes lying by the sides of the cephalic ganglia. Specimens protogynous.

Pregermarium beaufortiense new species.

Specimen 0.4–0.5 mm. long. Body spindle-shaped, with surface bearing densely crowded, short cilia. Pigment none, except for that of the four black eyes. These lie inside the cephalic ganglia which are enclosed by a dense, fibrous tunic. Small, refractive rhabdite-like bodies widely, but uniformly, distributed throughout the epidermis. Cephalic and caudal glands opening ventrally and subterminally. Pharynx plicatus, posteriorly directed, and communicating with a gonopharyngeal atrium and pore. Enteron sac-shaped, dorsal to other viscera (in life). No protonephridia. Single median ovary at anterior extremity of pseudocoele. Very short female genital canal receiving oocytes from pseudocoele. Two lateral vitellaria, which open into the sides of the female genital canal. Two rounded testes lying by and posterior to the cephalic ganglia. Nurse-cells, carrying maturing spermatozoa at their surfaces, sent into pseudocoele. Two posterior vasa deferentia. Muscular, unarmed, eversible penis communicating with the gonopharyngeal pore by way of the gonopharyngeal atrium. Protogynous. Chromosomes: X = 4, 2X = 8.

This species is described from the study of living specimens and from fixed and sectioned or totally mounted co-types. A total mount has been deposited in the United States National Museum (U.S.N.M. Cat. no. 20610) and others are in the collection of the Miller School of Biology (U. of Va. no. 877-1 and 877-2).

ANATOMY OF THE SPECIMEN

The short, spindle-shaped, colorless animal moves freely in the water by ciliary action. Scattered throughout the epidermis are somewhat irregular, rhabdite-like bodies (Fig. 1, *r*). The contours and the texture of these bodies resemble crystalloids rather than rhabdites. They should be considered pseudorhabdites.

There is but a single aperture in the body excepting the mouths of unicellular, epidermal glands. This is the gonopharyngeal pore (Fig. 1 and text figure 1, *gpp*), which is located on the mid-ventral line near the anterior level of the posterior fifth of the body.

The central nervous system is represented by a pair of dense, conspicuous, cephalic ganglia (Fig. 1, *g*). Each ganglion is covered by an intimately applied fibrous tunic. The nucleated bodies of the neurones crowd about the inner surface of this tunic. The ganglia are connected by a short commissure of transverse fibers.

Two eyes (Fig. 1, *ey*) are found *within* the fibrous region of each ganglion. The anterior eye is the smaller. Each eye consists of a black pigment-cup and one or more retinulae.[1]

No other organs of special sense were observed.

The "body-wall" is formed by an epidermis and a layer of circular and one of longitudinal muscles. The epidermal cells are not clearly defined. Indeed, the epidermis, in the fixed condition, resembles a syncytium with relatively few nuclei.

This "body-wall" encloses a pseudocoele within which the viscera and mesenchyme lie. The mesenchyme in the anterior and posterior thirds, and at the periphery of the middle third of the body is a typical parenchyma. The cells of the parenchyma in these three regions have, for the most part, reticular nuclei. About the axis of the middle third the cells of the parenchyma are few and there is present a sparse collenchyma or merely a perivisceral fluid. This sparse collenchyma permits the viscera to be shifted to a considerable extent.

The alimentary canal is unusual only in the position of its stout, highly muscular, plicate pharynx (Fig. 1, *ph*). It is posteriorly directed, from its mid-ventral attachment to the floor of the enteron, to open into the gonopharyngeal atrium. The sac-like enteron (Fig. 1, *e*), in life, lies dorsal to the other viscera. Its floor anterior to the pharynx tends to dip ventrally below the level of the plane in which the pharynx and penis lie. The enteron, which lies more or less free within the pseudocoele, may have its position shifted when the animal is fixed. In the fixed condition it is usually found lying ventral to the other viscera. The wall of the enteron is composed of a columnar epithelium and two layers of non-striated muscles.

No protonephridial system has been observed.

The specimens are protogynous.

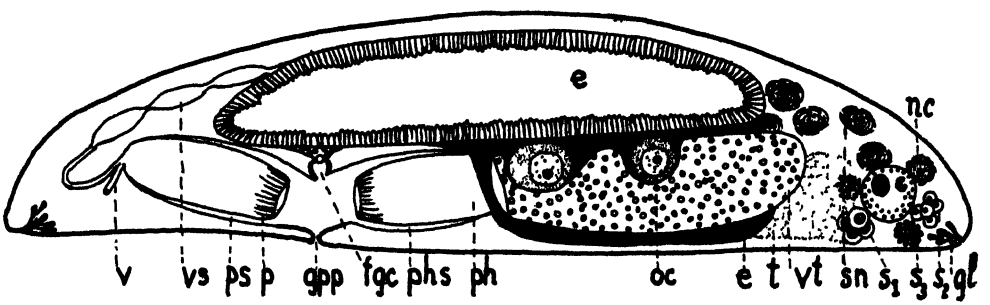
The female reproductive system is quite unusual. It involves a single gonad, part of the pseudocoele, a short, female genital canal, two large, laterally placed vitellaria and the gonopharyngeal atrium with its pore. The terminal ovary spends itself in the production of two series of oocytes (Fig. 1, *oc*), that stream out from the ovary over the cephalic ganglia into spaces beside the axis of the pseudocoele. In animals with an exhausted ovary only two rows of large oocytes may be found. These are on their way to a short, vertically disposed female

[1] Jameson (1897, p. 193) found that specimens of *Cylindrostoma quadrioculatum* "appear extremely sensitive to light."

genital canal (text figure 1, *fgc*). Spermatozoa are sometimes found within the female genital canal. Two lateral, club-shaped vitellaria (Fig. 1, *v*) lie beneath the series of large oocytes. These yolk-glands empty, through the walls of the female genital canal, into its lumen by means of short vitelloducts.

The male reproductive system involves two gonads, regions of the pseudocoele, two vasa deferentia, a stout, muscular, eversible penis, and the gonopharyngeal atrium with its pore.

Two club-shaped testes are to be found in specimens in which the female gonad had disappeared. Some of our observations suggest that spermatogonia may appear in the pseudocoele anterior to an ovary that is throwing oocytes into the pseudocoele. The manner in which the male gonads arise must be left for later investigation. A specimen, that is mature as a male, has two compact lateral testes (text figure 1, *t*) that lie somewhat by the side of and posterior to the cephalic ganglia. The spermatozoa in various phases of spermatogenesis



Textfigure 1. Reconstructed anatomy of *P. beaufortiense*. *e*, enteron; *fgc*, female genital canal; *gl*, cephalic glands; *gpp*, gonopharyngeal pore; *oc*, oöcytes lying within pseudocoele; *p*, penis; *ph*, pharynx; *phs*, pharyngeal sheath; *ps*, penis-sheath; s_1, s_2, and s_3 groups of cells representing phases in spermatogenesis; *sn*, spermatozoa about a nurse-cell; *t*, testis (left); *v*, vas deferens (right), and *vs*, mass of spermatozoa distending locally the vas deferens (left). × 333.

are sent out from these testes into the region anterior to the cephalic ganglia, in which the ovary had lain prior to its disappearance. From this terminal region groups of developing spermatozoa, each crowded about a nurse-cell (text figure 1, *nc*) travel back over the "brain" through the pseudocoele to the free ends of the vasa deferentia. By the time these masses of spermatozoa have reached the free ends of the vasa deferentia, they have consumed their nurse cells. The vasa deferentia (Fig. 1, *vd*) are thin-walled canals that receive masses of spermatozoa at their free anterior ends to carry them to the penis. The two ducts unite just before entering the penis. The anterior regions of the vasa deferentia may be seen, in life, to be distended locally by the presence of groups of spermatozoa within them. Near the penis these ducts become more muscular and have a greater caliber. Some authors have designated the anterior swollen regions of the vasa deferentia of species of *Cylindrostoma* to be false seminal vesicles. Others have found no vasa deferentia in *Cylindrostoma* (Reisinger, 1926, p. 449).

The penis (Fig. 1, and text figure 1, *p*) is a conspicuous feature of *P. beaufortiense*. It appears in life to be a stout tubular, muscular organ with its axis lying somewhat ventral and parallel to that of the pseudocoele. Sections show it to be a doubly inverted tapering tube with a club-shaped head at its free end (Fig. 3). There are no cuticular structures arming this peculiar penis. Its head, however, possesses a heavy, complex musculature (Fig. 3, *mu*). The lumen of the penis winds irregularly through the head of the withdrawn penis.

The penis, preparatory to being projected through the gonopharyngeal pore, may drag its base antero-dorsally through from 90° to 120°. This rotation shifts the position of the enteron and vitellaria. The penis, enteron, and vitellaria are usually shifted in this manner in fixed specimens.

The penis delivers spermatozoa to the female genital canal, for we have found spermatozoa in this duct. The penis, however, functions in another manner. We have observed it to dart from the gonopharyngeal pore to wound another specimen by sending a packet of spermatozoa into the latter's pseudocoele.

Pregermarium caroliniensis new species

Specimen 0.3–0.4 mm. long. Body spindle-shaped. Surface densely crowded with short cilia of uniform length. Two strands of black pigment lie over the ganglia near the mid-line. These bands of pigment extend from the anterior margin of the enteron to the anterior end of the pseudocoele. These and the four pigment-cups of the eyes represent the only pigment present. The four eyes lie within the dense, fibrous membrane that invests the "brain." Small, refractive rhabdite-like bodies are scattered widely, but uniformly, throughout the epidermis. Cephalic and caudal glands open ventrally and subterminally. A slender, plicate pharynx, directed posteriorly, communicates with the gonopharyngeal pore. Enteron sac-shaped, dorsal (in life) to other viscera. No protonephridia. Single, median ovary at anterior extremity of pseudocoele. Oocytes delivered to pseudocoele. Very short female genital canal, opening into pseudocoele. Two lateral vitellaria opening into sides of the female genital canal. Two compact testes lying by and posterior to the cephalic ganglia. Nurse-cells, carrying maturing spermatozoa, at their surfaces, sent into pseudocoele. Two posterior, heavy-walled vasa deferentia. Penis, in contracted condition, short, nearly spherical, unarmed, communicating with the gonopharyngeal pore. Protogynous. Chromosome number: X = 4; 2X = 8.

This species is described from many co-types, some of which have been preserved as total mounts, others sectioned. A total mount has been deposited in the United States National Museum (U.S.N.M. Cat. no. 20611). Total mounts and section specimens are in the collection of the Miller School of Biology (U.Va. no. 878–5, 878-10, and 878-12).

ANATOMY OF SPECIMEN

The short spindle-shaped animal moves freely in the water by means of a dense coat of uniform cilia. The epidermis bears rhabdite-like, rod-shaped bodies (Fig. 4, *r*). These pseudorhabdites resemble rhabdites more than do those of *P. beaufortiense*.

Conspicuous, sub-epidermal glands open sub-terminally and ventrally at the

anterior and posterior ends of the body. The posterior glands converge behind the penis to form a poorly defined reservoir-like region, that is sometimes to be seen in a living specimen as an opaque, colorless, oval mass. It is, however not a receiving vesicle. These posterior glands may suddenly discharge a refractive viscous substance that anchors the specimen to a region of the substratum. The animal may then drag itself from this place of anchorage to be yet held by a growing thread of refractive, viscous material (Fig. 4, *m*). This substance and the method in which it is used suggests the structure and function of byssus material of Mollusca.

A gonopharyngeal pore lies in the mid-ventral line of the body near the anterior level of the posterior fifth of the body.

The central nervous system presents a pair of conspicuous cephalic ganglia (Fig. 4, *g*). Each ganglion is covered by an intimately applied fibrous sheath. The nuclei of the neurones crowd about the inner surface of this sheath. The ganglia are joined by a stout commissure (Fig. 5, *co*) of transverse fibers.

A pair of eyes (Figs. 4 and 5, *ey*) lies within the fibrous region of each ganglion. Each eye consists of a black pigment-cup and one or more retinulae.

No other organs of special sense have been observed.

The "body-wall" is formed by an epidermis and a layer of circular and one of longitudinal muscles. A set of very heavy longitudinal muscles arise from the posterior, ventral region to be inserted upon the pharynx as the latter's retractor muscles. These retractor muscles appear to be but a set of the general longitudinal elements that have been greatly enlarged.

The pseudocoele, enclosed by the "body-wall," bears mesenchyme and viscera.

The mesenchyme, in the anterior and posterior regions, is a typical turbellarian parenchyma, there being many cells that bear reticular nuclei (Fig. 3, *pc*). However, these cells become fewer—rather abruptly—axially in the middle two-thirds of the pseudocoele. Here the mesenchyme is a sparse collenchyma rather than a parenchyma. This permits extensive shifting of the viscera.[2] Two strands of black pigment (Fig. 4, *pg*) extend anteriorly from the enteron over the ganglia to the anterior limit of the pseudocoele.

The peculiar feature of the alimentary canal is the posteriorly directed pharynx (Fig. 4, *ph*). This is a slender muscular organ of the plicate type. Ten nuclei (Fig. 4, *nu*) are to be seen as refractive spheres within the wall of a living pharynx. This cylindrical pharynx extends from the mid-ventral region of the living enteron to a point beyond its posterior margin. The pharynx has power of great distention. The sudden ejection of the elongating pharynx (Fig. 6, *ph*) from the gonopharyngeal pore surprises the observer. This ejection involves both the elongation of the pharynx and the foreshortening of the body. The sac-like enteron's floor, anterior to the pharynx, dips ventrally between the

[2] The two series of oöcytes, in living animals, lie between the ventral vitellaria and the dorsal enteron. The penis lies in the axis of the body and is directed anteriorly in living forms. In fixed specimens, the enteron has been crowded down between and beneath the series of oöcytes and vitellaria. These viscera have been shifted, apparently, by the penis having been thrown dorsally through 90 or more degrees when the specimens were fixed.

lateral vitellaria. The wall of the enteron is composed of a columnar epithelium[3] and two layers of muscles.

No protonephridial system has been observed.

The specimens are protogynous.

The female reproductive system involves a single ovary, two paths in the pseudocoele (along which oöcytes are carried), a short dorso-ventrally disposed female genital canal, two lateral vitellaria, and a gonopharyngeal atrium with its pore. The ovary (Fig. 5, *o*) extends from the anterior region of the pseudocoele to the "brain." Oögonia are given off from its posterior region to drift over the neural commissure into the pseudocoele to form two series of oöcytes (Fig. 4, *oc*). Thus the oöcytes as two series (a right and a left) travel through the pseudocoele to the female genital canal.

As the ovary spends itself in the elaboration and discharge of oöcytes, two lateral male gonads (text figure 1, *t*) make their appearance[4] by the sides of the cephalic ganglia. These testes give off groups of cells that present various phases of spermatogenesis. Each group of developing spermatozoa is formed about a nurse-cell. The region anterior to the "brain" contains many spermatozoa in various stages of development. This region, therefore, is concerned with the development of both male and female gametes. The groups of spermatozoa (Fig. 4, *sn*) are sent back over or by the "brain" along two lateral paths within the pseudocoele. By the time they enter the free ends of the two vasa deferentia, they have reached maturity and have lost their nurse-cells. The vasa deferentia (Fig. 4, *vd*) are heavy, muscular, cylindrical ducts that unite as they enter the base of the penis. The penis, in the living specimen, is a muscular, spherical organ. It has a remarkable faculty to distend itself. An animal, that is held under slight coverglass-pressure, may first thrust out its pharynx (Fig. 6, *ph*) and next its penis (Fig. 6, *p*). The latter may suffer autoamputation of its base. The pharynx has not been seen to break from the body. No armature has been observed upon this eversible copulatory organ.

Pregermarium beaufortiense	*Pregermarium caroliniense*
0.4–0.5 mm. long	0.3–0.4 mm. long
pigment only in pigment cups of four eyes	pigment in two streaks as well as in pigment cups of 4 eyes
caudal glands inconspicuous	caudal glands conspicuous, elaborating mucus-like matter
vasa deferentia thin-walled	vasa deferentia thick-walled
penis cylindrical	penis spheroidal
pharynx cylindrical, with wide diameter	pharynx cylindrical, slender, with 10 refractive nuclei, 5 on each side
resting pharynx does not extend posteriorly beyond enteron	resting pharynx extends posteriorly beyond enteron

[3] Böhmig, (1890, p. 68) describes the linings of the entera of *Cylindrostoma klostermanni* and *quadrioculatum* as epithelia.

[4] Some of our slides suggest that spermatogonia and spermatocytes are present in the anterior extremity of the pseudocoele before the ovary has spent itself. Material will be collected next summer in order to carry out this suggestion.

DISCUSSION

The genus *Cylindrostoma* Oersted appears not to be so well defined as it should be. Graff (1880) recognized it, whereas later (1913) he considered it to be a pseudonym for *Pseudostomum* Schmidt. Bresslau (1933), however, recognizes it as the only genus belonging to the sub-family, Cylindrostominae. Reisinger (1926) described two new species: *Cylindrostoma canhoffeni* and *gaussi*. All authors except Levinson (1879) have described the testes of their species of *Cylindrostoma* as being vesicular. Levinson, in describing three new species, indicated that two of them had vesicular testes. His *C. discors*, however, is stated to have two ovate testes. Böhmig (1890, p. 12) does not recognize the compact or ovate testes of Levinson's *C. discors*, for he says that the testes of all *Cumulata* are vesicular. Reisinger (1926) refers to "Hodenfollikel" in the anterior region lying over and about the sides of the "brain" of his *C. canhoffeni* and *gaussi*. These Hodenfollikelen, lobes of spermatozoa, or testes-follicles are evidently comparable to the masses of spermatozoa, grouped about nurse-cells, that we have observed in our two species. Indeed, Reisinger, himself, refers to the "Hodenfollikel" of his specimens as having in each a "Cytophor" that is apparently of a cellular nature. This suggestion, on the part of Reisinger, that the follicles of the testes may not be after all follicles but groups of developing spermatozoa makes it doubtful that Reisinger's two species could be included in the sub-order *Cumulata*, the members of which are described as having vesicular testes.

The uncertainty concerning the condition of the testes in Reisinger's species, prepares the ground for the reception of our two *Cumulata* that have compact testes, as do all rhabdocoele-genera except two, *Alaurina* and *Mecynostoma* (Böhmig, 1890, p. 12). We hold that the *Cumulata* should be defined as having vesicular testes except for some species of *Cylindrostoma* and *Pregermarium beaufortiense* and *caroliniense*.

A second characteristic of the genus *Cylindrostoma* is that all of its species bear two germovitellaria or two ovaries and two vitellaria. Reisinger (1926) held that his *C. canhoffeni* and *gaussi* had two club-shaped, lateral vitellaria. His figures, however, show, with figures of some other species of *Cumulata*, details that are not convincing. The oöcytes of the germovitellaria in these figures are indicated to be nearly equal in size. They do not grade off into small oögonia as would be expected to be the case in the germinal region of a germovitellarium. We, too, had inferred that our two forms belonged to *Cylindrostoma*. We searched diligently in the living forms for a series of cells, within what we considered to be the germovitellarium, that ranged from large oöcytes to small oögonia. Our search became more pronounced when our sections indicated that the oöcytes were not, after all, parts of germovitellaria, but were lying free within the pseudocoele. The situation was finally explained by our discovery that these specimens are protogynous and that there is a single ovary within the pseudocoele anterior to the "brain." We expect that further study will reveal that Reisinger's (1926) *C. canhoffeni* and *gaussi* will likewise be protogynous, and have a single ovary in the "head." The *Cumulata* must now

be defined as a sub-order that may have either germovitellaria, ovaries and vitellaria, or an ovary and vitellaria.

Our specimens are included in a new genus, *Pregermarium*. This genus belongs to the sub-order *Cumulata* (=*Holocoela*), the family Cylindrostomidae, and the sub-family Cylindrostominae (Bresslau, 1933).

Our animals display some habits and many features that suggest the genus *Cylindrostoma*.

They live among algae. Jameson (1879, p. 173) found "a few specimens of *C. quadrioculatum* (Leukart)" "among *Cladophora*, collected in shallow rock pools, near high water mark." Sabussov (1905) found the same species, together with *C. klostermanni* Jens., living in masses of marine algae.

Pregermarium beaufortiense, *P. caroliniense*, *Cylindrostoma klosmanni* and *Plagiostoma philippense* are exceptional alloeocoeles in that they bear rhabdites or rather pseudorhabdites.

Our two forms resemble plagiostomids and polyclads in that their "brains" are enclosed in a fibrous sheath.

They also display, in common with other rather closely related forms, the faculty of injecting spermatozoa hypodermically into the pseudocoele of their fellows. The injection of spermatozoa by one individual into another at a region remote from the female genital canal has been recorded by Hyman (1938) in *Hydrolimax grisea* and by Kepner, Stirewalt, and Ferguson (1941) in *Plagiostomum dahlgreni*. We have also observed this in *P. beaufortiense*. These observations must not lead to the inference that the female genital canal serves only as a passage way for ova. The female genital canal of *P. beaufortiense* is much more vestige-like than that of either *Hydrolimax grisea* or *Plagiostomum dahlgreni*. Despite this we have a section of this small canal, on slide 877-1, that shows it containing spermatozoa. The female genital canal, therefore, may function as a copulatory duct.

The parenchyma, too, suggests that of other closely related forms. Böhmig (1890), Hyman (1938), and Kepner, Stirewalt, and Ferguson (1941) have called attention to the peculiar parenchyma that lies about the axis of the middle third of the bodies of different *Cumulata*. This mid-region of the pseudocoele of our two new species has become more conspicuous in that the viscera may be shifted within it rather extensively, indicating the presence of a perivisceral fluid rather than either a parenchyma or a collenchyma.[5]

Whether there be a collenchyma or a perivisceral fluid in this mid-region of the pseudocoele, the fact remains that maturing male and female germ-cells pass through the mid-pseudocoele on their way to the mouths of their respective gonoducts.

Herein, we have a feature of these alloeocoeles that resembles that of the acoeles. Meixner (1938, p. 48–9) says that there is usually in the acoeles a pair of germinal centers in the anterior region of the body from which ova grow out into the parenchyma as free cells.

The passage of ova through the pseudocoele suggests the manner in which

[5] Pereyaslawzewa (1892) referred to a perivisceral fluid in an *Aphanostoma*.

ova travel from gonad to gonoduct through the coelom of coelomatous animals. This is not the first time that the coelom of turbellaria has brought to mind the coelom of coelomatous animals. Kepner, Ferguson, and Stirewalt (1938) observed that the wall of the receptaculum seminalis of *Mesostoma virginiana* is covered by an epithelium which suggests the splanchnic peritoneum's epithelium of coelomatous animals.

Thus the acoelomatous turbellaria have two features in their pseudocoele that resemble two features of the coelomatous animals' coelom.

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EXPLANATION OF PLATE 12

Figure 1. Dorsal aspect of *P. beaufortiense*. Most of the epidermis and half of the enteron are shown as having been dissected away. *e*, enteron; *ey*, anterior, smaller eye (right); *g*, cephalic ganglion (right) *gpp*, gonopharyngeal pore; *ng*, nidamental glands associated with lips of gonopharyngeal pore; *oc*, oocytes lying within pseudocoele; *p*, penis; *ph*, pharynx; *ps*, packet of spermatozoa implanted in epidermis by another individual; *r*, rhabdite; *sn*, mass of spermatozoa about a nurse-cell; *v*, vitellarium (right), and *vd*, vas deferens (left). × 333.

Figure 2. Meiotic chromosomes of *P. beaufortiense*. × 2000.

Figure 3. Longitudinal section of penis of *P. beaufortiense*. *mu*, oblique and circular nucleus of head of penis, and *pc*, reticular nucleus of a parenchyma cell (Slide 877-1). × 466.

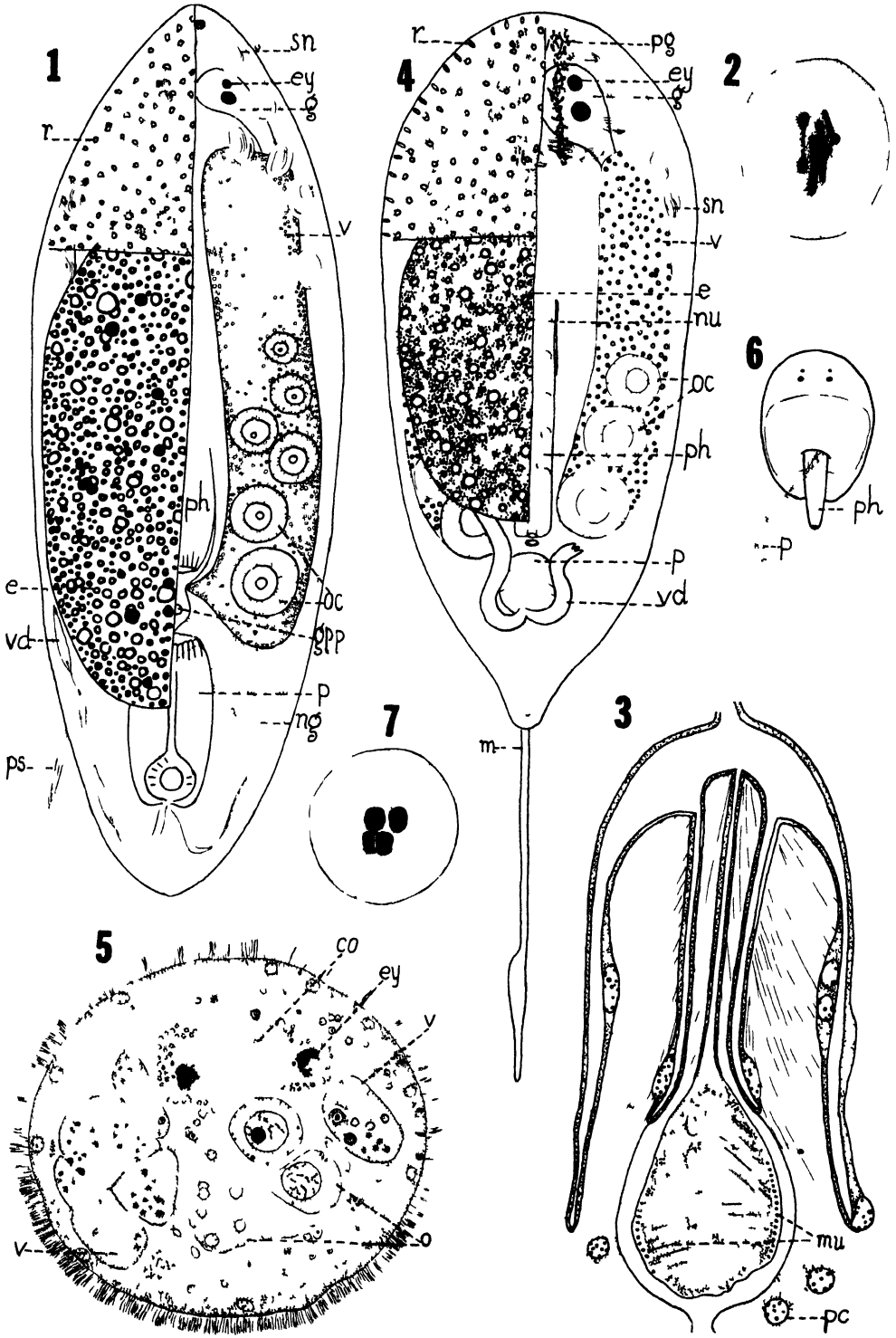
Figure 4. Dorsal aspect of *P. caroliniense*. Most of the epidermis and half of enteron shown as having been dissected away. *e*, enteron; *ey*, anterior, smaller eye (right); *g*, cephalic ganglion (right); *m*, anchoring strand of viscous secretion of caudal glands; *nu*, one of the ten nuclei that lie within the wall of the pharynx; *oc*, oöcytes lying within the wall of the pharynx; *p*, penis; *pg*, band of mesenchymal pigment (right); *ph*, pharynx; *r*, rhabdite; *sn*, mass of spermatozoa about a nurse-cell; *v*, vitellarium, and *vd*, vas deferens (right) indicated cut off to show posterior limit of vitellarium. × 333.

Figure 5. Transverse section of *P. caroliniense*. *co*, neural commissure; *ey*, eye; *o*, germarium or ovary; and *v*, vitellaria (Slide 878–5). × 233.

Figure 6. Ventral aspect of *P. caroliniense* showing penis (*p*), and pharynx (*ph*) thrown out through the gonopharyngeal pore. × 60.

Figure 7. Meiotic chromosomes of *P. caroliniense*. × 2000.

PLATE 12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NEW LITHOBIID CENTIPEDES FROM NORTH CAROLINA[1]

By Nell Bevel Causey

Two Text Figures

The centipedes described in this paper were collected by Mr. James H. Starling from the Duke Forest, Durham, North Carolina, in the fall and winter of 1941–1942. Since their small size, swift movement, death-feinting instinct, and coloration make collection by ordinary methods difficult, a modified Berlese funnel was used. The type specimens have been sent to the Museum of the Academy of Natural Sciences of Philadelphia.

SERROBIUS n. gen.

This genus resembles *Neolithobius* in the modification of the fourth segment of the anal legs of the male. That segment is excavated dorsally and conspicuously elevated at both ends. *Serrobius* can be distinguished from *Neolithobius*, which has the third segment of all anterior legs armed dorsally with three spines, by the presence of one, two, or rarely three dorsal spines on the third segments of the legs anterior to the twelfth.

Genotype.—*S. pulchellus* n. sp.

Serrobius pulchellus n. sp.

General *color* light brown, head darker. *Antennae* composed of from 26 to 29 articles; their length is equal to the distance from the front margin of the head to the base of the fifth pair of legs. *Ocelli* eight, sometimes nine, in three series of 4, 3, 1. Prosternal *teeth* from 4–4 to 5–6, median incision U-shaped, line of apices recurved, ectal spines hair-like. Posterior corners of *dorsal plates* 11, 13, and 15 weakly produced; plates smooth but bearing a few short setae. *Coxal pores* 3(2), 3, 3, 2(3); circular. *Gonopods* of male uniarticulate, each bearing two long setae. Claw of female gonopods moderately long and stout, excavated mesally, outer and inner teeth small and indistinct; two basal spines, the inner but slightly shorter than the outer, both of uniform width the proximal third or half of length, then narrowed to an acute point, the margins slightly serrulate. *Spines* on legs as follows: first, $\frac{0,0,1,1(2),1}{0,0,1(2),1(2),1}$; second through fifth, $\frac{0,0,2,2,1(2)}{0,0,2,2,1}$; sixth through tenth, $\frac{0,0,2(3),2,2}{0,0,1(2),2,2}$; eleventh, $\frac{0,0,2,3,2}{0,0,2,2(3),2}$; twelfth, $\frac{1,0,2,1,1}{0,1(0),3,3,2}$; thirteenth, $\frac{1,0,2(3),1,1}{0,1,3,3,2}$; fourteenth, $\frac{1,0,3,3,1}{0,1,3,3,2}$; fifteenth, ♂, $\frac{1,0,5\text{–}8,1,0}{1,0,3,3,2}$; ♀, $\frac{1,0,4,1,0}{0,1,3,3,2}$. Fourth segment of anal legs of male modified as shown in figure 1; distal end of third segment of anal legs of male inflated. Penult legs with no special modifications. Terminal *claws* of last two pairs of legs single; on all other legs there are two small accessory claws in addition to the usual large terminal claw. Many spines are serrulate distally. All *tarsi* divided, but flexion of anterior tarsi

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limited. *Length*, 9.6–11 mm. Penult legs about 3.6 mm. and anal legs about 3.2 mm. Width and length of head plate approximately equal. Body length 9.3 times width of tenth dorsal plate.

PEARSOBIUS n. gen.

Resembles *Neolithobius* and *Serrobius* in having the fourth segment of the anal legs of the male enlarged and excavated distally. Differs from those genera in that no dorsal plates are produced, the third segments of the anterior legs are armed dorsally with two spines rather than with one or with three, and the *coxae* of the *anal legs* are *ventrally armed*. *Zinapolys*, with the coxae of the last two pairs of legs ventrally armed, is the only other American genus of the

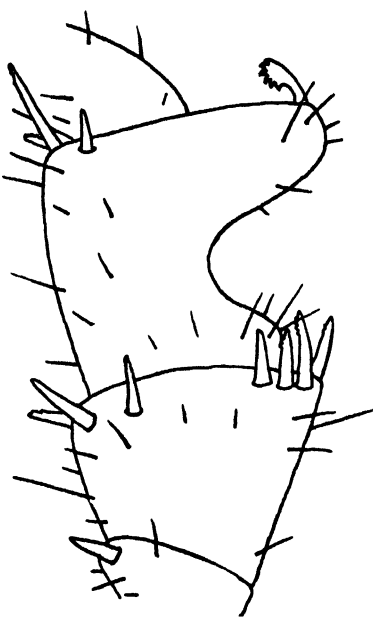

FIG. 1. *Serrobius pulchellus* n. sp. ♂ . Lateral view of third and fourth segments of anal leg. × 70

Lithobiomorpha, outside of the Ethopolidae, with any of the coxae ventrally armed.

Genotype.—*P. carolinus* n. sp.

Pearsobius carolinus n. sp.

Color light brown; head and antennae darker. *Antennae* of type specimen broken, but they are known to consist of more than 27 articles and to be longer than the distance from the front margin of the head to the base of the sixth pair of legs. *Ocelli* about 9 or 11, indistinct, in four series. Prosternal *teeth* 5–5, line of apices recurved, median incision a deep U, ectal spines hair-like. No

dorsal plates are produced, but the posterior margins of 15, 13, and 11 are slightly recurved. The smooth head and dorsal plates bear a few setae of variable length. *Coxal pores* 3, 4, 4, 2; circular. *Gonopods* of the type specimen, a male in the *immaturus* stage, uniarticulate and glabrous. *Spines* on legs as follows: first and second, $\frac{0,0,2,2,1}{0,0,2,2,1}$; third, $\frac{0,0,2,2,2}{0,0,2,3,1}$; fourth through ninth (sixth lost), $\frac{0,0,2,2,3}{0,0,2,3,1}$; tenth and eleventh, $\frac{0,0,3,2,3}{0,0,3,3,1}$; twelfth, $\frac{0,0,2,2,2}{0,0,3,3,2}$; thirteenth, $\frac{1,0,2,1,1}{0,1,3,3,2}$; fourteenth, $\frac{1,0,3,1,1}{0,1,3,3,2}$; fifteenth, $\frac{1,0,3,1,0}{1,1,3,3,2}$. The first through the thirteenth legs bear three terminal *claws*, two of which are small; tarsi of the last two pairs of legs bear single terminal claws. Many spines are serrulate distally. Fourth segment of anal legs of male modified as shown in figure 2. *Tarsi* of all legs divided, but the division is very faintly indicated on the anterior tarsi. *Length*,

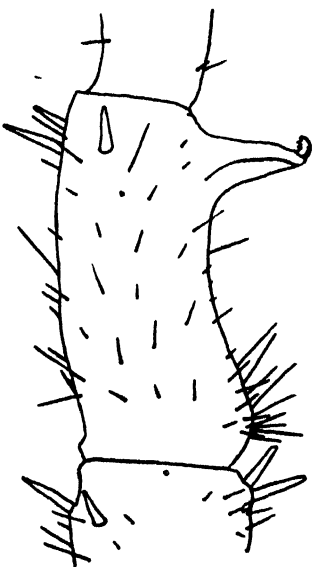

FIG. 2. *Pearsobius carolinus* n. sp. ♂. Lateral view of fourth segment of anal leg. × 80

10 mm. Length of anal and penult legs 3.7 and 3.5 mm. respectively. Ratio of length of head plate to its width 27:29. Body length 7.7 times greater than the width of the tenth dorsal plate. Only the type specimen is known.

Sigibius starlingi n. sp.

Resembles *Sigibius nidicolens* Chamberlin 1938 in size and in the dorsal spining of the posterior legs. In *starlingi* the ventral spines on the fifteenth and fourteenth legs are 0, 1, 3, 1, 0, and 0, 1, 2, 1, 0, respectively; whereas, in *nidicolens* the spines on the corresponding legs are 0, 1, 1, 1, 0. Coxal pores in *nidicolens* are 2, 2, 2(3), 2 and in *starlingi* 1, 1, 1, 1(2).

Color amber, head darker. *Antennae* consist of 24 articles; their length

equals the distance from the front margin of the head to the base of the fourth pair of legs. *Ocelli* 1 + 2, 2, the single ocellus but little larger than the others. Prosternal *teeth* 2–2, median incision a deep V, line of apices straight, distance between apices one-half greater than width of incision, ectal spines more prominent than prosternal setae. Posterior corners of *dorsal plates* 15, 13, 11, and 9 slightly produced; plates and head smooth; strong setae regularly spaced along lateral and posterior margins of plates, and smaller setae thinly scattered over head and plates. *Coxal pores* 1, 1, 1, 1(2); circular, small. *Gonopods* of male uniarticulate, with no setae or bristles. *Spines* on legs as follows: first, $\frac{0, 0, 0, 0, 1}{0, 0, 0, 0, 0}$; second through fifth, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 1}$; sixth through eleventh, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 1, 1}$; twelfth and thirteenth, $\frac{0, 0, 1, 1, 0}{0, 0, 1, 1, 1}$; fourteenth, $\frac{0, 0, 2, 1, 0}{0, 0, 2, 1, 0}$; fifteenth, $\frac{0, 0, 2, 0, 0}{0, 1, 3, 1, 0}$. Only the *tarsi* of the last two pairs of legs are biarticulate; division of the tarsi of the thirteenth and fourteenth legs is faintly evident, but division of other tarsi is doubtful. All tarsi terminate in three *claws*, the usual large one and two small accessory claws. Posterior legs of the male show no special modification other than the usual swelling. *Length* of male type specimen 5.1 mm. Length of fifteenth and fourteenth legs 1.67 and 1.22 mm. respectively. Ratio of length of head plate to its width 34:31. Body length 11 times greater than the width of the tenth dorsal plate. Ony the type specimen is known.

Nampabius (Carolobius n. subgen.) **pinus** n. sp.

Placed in a new subgenus on the basis of the lack of a lobe, which is present in males of other species of *Nampabius*, on the fifth segment of the penult legs. *Color* amber; head and antennae darker. Length of *antennae* equals distance from front margin of head to base of third pair of legs; each of the two known specimens had one antenna of 18 articles and the other of 20. *Ocelli* in two series, 3, 2; pale and irregular in size and shape. Prosternal *teeth* 2–2, pale, medial incision a deep V, line of apices straight or but slightly recurved, ectal setae prominent. Posterior angles of *dorsal plates* 14, 12, and 10 slightly produced; a long seta on each of the posterior corners of most plates, and shorter setae thinly scattered over head and plates. *Coxal pores* 1, 2, 2, 2; circular, small. *Gonopods* of male small, uniarticulate, each with two long bristles. All anterior legs bear a single small and inconspicuous dorsal spine at the distal end of the femur and of the tibia. More conspicuous *spines* are as follows: first through sixth, $\frac{0, 0, 0, 0, 0}{0, 0, 0, 0, 1}$, 3 claws; seventh through thirteenth, $\frac{0, 0, 0, 0, 0}{0, 0, 0, 1, 1}$, 3 claws; fourteenth, $\frac{0, 0, 1, 0, 0}{0, 1, 2, 1, 1}$, 3 claws; fifteenth, $\frac{0, 0, 2(1), 0, 0}{0, 1, 2, 1, 0}$, 1 claw. Posterior legs show no modification other than the usual swelling. *Tarsi* of only the last two pairs of legs divided. *Length* of type and of a cotype, 4.6 and 4.4 mm., males. Length of anal and penult legs 1.3 and 0.9 mm., respectively. Length and width of head plate equal. Body length 9.8 times greater than the width of the tenth dorsal plate.

Lianobius chamberlini n. sp.

Color light amber, head darker. *Antennae* composed of 17 articles; their length equals the distance from the front margin of the head to near the base of the third pair of legs. *Ocelli* light and varying considerably in size; in the type there are three in one series on one side of the head and four in two series on the other side; mostly four in cotypes. Prosternal *teeth* 2–2, lines of apices straight, median incision deeply V-shaped. Posterior corners of *dorsal plates* 14, 12, and 10 produced; head and plates bear a few setae, the longest of which are on the posterior corners of the plates. *Coxal pores* 1, 1, 1, 1; in one of the cotypes 1, 2, 1, 1; circular. *Gonopods* of male small, inarticulate, each with two long bristles. *Spines* on legs of the type as follows: first through fifth, $\frac{0,0,0,0,1}{0,0,0,0,1}$, but $\frac{0,0,0,0,1}{0,0,0,1,1}$ on the fourth and fifth of a cotype; sixth through ninth, $\frac{0,0,0,0,1}{0,0,0,1,1}$; tenth through thirteenth, $\frac{0,0,0,0,0}{0,0,0,1,1}$; penult and anal, $\frac{0,0,0,0,0}{0,0,1,1,0}$. Anal and penult legs terminate in a single tarsal claw; other legs terminate in three claws. *Tarsi* of only the two posterior pairs of legs divided. Posterior legs of the male show no special modification other than the usual swelling. *Length* 4.4 mm. Length of fifteenth and fourteenth legs 1.19 and 0.80 mm. respectively. Width and length of head plate approximately equal. Body length 11 times width of tenth dorsal plate.

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INFLUENCE OF TEMPERATURE ON SIZE AND FORM OF *CYCLOPS VERNALIS* FISCHER

By Dorothy Aycock

Ten Text Figures

INTRODUCTION

For a long time investigators have noted the diversity in size of adult copepods at different seasons of the year and in different waters, and this variation has often been attributed to food supply or to growth after sexual maturity, although no direct observational evidence of post-larval growth has been adduced. Only recently have specific experiments been conducted to ascertain the influence of environmental conditions on size and form of copepods.

Lilljeborg (1901) was one of the first to note diversity in size of copepods at different seasons. He found spring and summer forms in *Cyclops strenuus*, a larger spring form giving rise to a smaller summer form. He recognized 5 different forms or varieties of *Cyclops strenuus*, some of which were seasonal in occurrence. Hartmann (1917) found that *C. strenuus*, and several species of Diaptomus were somewhat larger in winter than in summer, and attributed this condition to age. He also found minor seasonal differences: for *C. strenuus*, in lengths of setae and of the second segment of the fifth foot; and, for certain species of Diaptomus, in form of the hyaline plate of the antepenultimate segment of the clasping antenna of the male and in armature of the 5th foot of the female. He characterized these diversities as temporal variations, but there was no evidence that the copepods he compared had developed at different seasons, and he used size as a criterion of age. Hartmann believed that there was a post-larval growth of the animals, and mistakenly regarded larger copepods as being merely older.

Russell (1928), in a study of the behaviour of *Calanus finmarchicus*, found that Calanus born in the early spring when the sea temperature was low gave rise to a generation of large adults, which in turn yielded generations of smaller individuals born when the temperature of the water was rising rapidly. He made no winter observations, but concluded that some of the small individuals produced in late summer carried over until the spring breeding season.

Coker (1933) reviews the contributions of several other observers; his own work is mentioned in a following paragraph.

Bogorov (1934) made an extensive study of the seasonal changes in Calanus, and concluded that size was greatest at low temperatures, and vice versa. Marshall, Nicholls and Orr (1934) found the same correlation.

Clarke and Zinn (1937), on the other hand, state that hauls of Calanus taken in May contained small adults and that these animals were from the new spring generation born at the end of April or early in May when the temperature ranged between 5° and 9°C. The generation produced in June at temperatures of 10° to 15°C. apparently did not reach maturity until the following January or February. In consequence of the longer period of growth, the authors seem to

imply, the summer-bred generation comprised larger adults than the spring generation. This relationship is just the reverse of that reported by Marshall, Nicholls and Orr, and by Bogorov. Clarke and Zinn add, however, that the large adult females taken in April may have been spawned in March, and, if so, present a case of correlation of large size and low temperature during the spawning period.

It is evident from the foregoing discussion that there are abundant data regarding the occurrence of diversity in size of copepods, sometimes accompanied by minor differences in form, but Coker (1933) seems to have been the first to demonstrate experimentally the correlation of size and form with a particular environmental condition—temperature. In his experiments he employed *Cyclops serrulatus*, *viridis*, and *vernalis*, with emphasis on the last-mentioned species. The experiments with *vernalis* were conducted with the progeny of one female. Nauplii were placed at three distinct temperature levels—28–30°, 18–19°, and 7–10°C. Animals reared at the lowest temperatures were invariably much larger than those reared at the highest temperatures, thus establishing an inverse correlation of size with temperature. In order to ascertain whether the difference in size attained at high and low temperatures was due to differences in food supply, or whether it was attributable primarily to the influence of temperature on the animals, experiments were conducted in which an extremely deficient food-supply was used. The result was retardation or arrest in development, with no notable degree of dwarfing, the copepods still attaining approximately the maximum size for the line and the temperature. In conclusion, it is stated that "all of the experiments with reduced food-supply tend to negative the idea that the appearance of correlation of size with temperature could have been due to the influence of temperature on food-supply in the cultures, rather than to its control of internal processes" (p. 433).

The same author (1934a) considered also the influence of temperature on the form of *Cyclops vernalis*. There was evidence that "length of furcal rami relative to total length of body (exclusive of furcal setae) is inversely correlated with the temperature at which the copepod is reared; but the coefficient of correlation (undetermined) is evidently small and the correlation so obscured by wide individual variation that it would not ordinarily be observable" (p. 420). As to the proportions of the furca, he states that "the width of furcal rami, relative to length of the rami, is directly correlated with temperature of rearing: thus, the furcal rami of copepods reared at high temperatures (28–30°) are conspicuously wider, relative to length, than those of copepods reared at low temperatures (7–10°)—approximately $\frac{1}{3}$ wider in females and $\frac{1}{4}$ wider in males" (p. 421). There were, furthermore, "indications of a tendency to reduction in the number of spines on the terminal segments of the exopods of the swimming feet, when the copepods are reared at high temperatures. The observations are inadequate for conclusion" (p. 421).

Data assembled by the present author with respect to *C. vernalis* Fischer bear on several questions presented in the preceding paragraphs, particularly, on spine formula in relation to temperature.

MATERIALS AND METHODS

All animals used in the experiments had known parentage, both parent copepods having been reared in the laboratory. The line was originally begun by the collection of a female with egg sacs, mated in the wild, and collected October 26, 1938, from a small pool in the vicinity of Chapel Hill, N. C. Nauplii from the first pair of egg sacs shed by this wild female were placed by twos in shell vials, and the first of these to develop egg sacs gave rise to the copepods used in the experiments. Each clutch of newly hatched nauplii was divided, half being placed at 28.1°C. and half at 7.7°C. The temperatures at which the experiments were carried out were accurately controlled by the use of the constant-temperature apparatus described by Coker and Constable (1936). Females with egg sacs that were used in the experiments were selected from a mass culture kept at room temperature. Later on during the course of the experiments, some of the females were selected from the 28.1° cultures. Copepods upon which observations were made were reared each in a separate vial, rather than in mass cultures which might have yielded greater numbers with less assurance of adequate food supply for every copepod.

The food used consisted principally of rich manure cultures of miscellaneous Protozoa and pure culture of *Chlorella pyrenoidosa* in an organic medium.* The protozoa furnished the base for the food supply, but the Chlorella added from time to time seemed necessary to general good health and fertility. Twice in course of the experiments the supply of Chlorella was exhausted, and each time there was an obvious decrease in activity and fertility of the animals. The number of drops of food added to the cultures depended, of course, on the satisfactoriness of the infusion being used and the number of animals being fed. Food was added until examination of a drop of the culture medium under the microscope showed a bountiful food supply for the animals. After the culture was first established, subsequent examinations were made on an average of once a week, and more food added if needed. Chlorella was usually added in a proportion of one drop to five of manure culture.

OBSERVATIONS

Size (see figs. 1 and 2)

The most evident effect of temperature on *Cyclops vernalis* was the difference in size of animals reared at high and low temperatures. Length was measured from "brow" to tip of furcal rami. The mean length of body of 37 females reared at 28.1° was 1.063 mm. (± 0.066), with lengths ranging from 0.889 to 1.289 mm.; the mean for 23 females reared at 7.7° was 1.517 mm. (± 0.061), with lengths ranging from 1.158 to 1.671 mm. The overlapping in the two groups is caused only by two particularly small low-temperature copepods, measuring respectively 1.254 mm. and 1.158 mm. All other animals in this group were much longer, the third smallest measuring 1.412 mm. The results, and the very figures, are close to those obtained by Coker (1933) with European copepods of

* The original stock of Chlorella and directions for culturing were kindly furnished by Dr. B. M. Duggar.

the same species, but obtained from waters in Paris, when 199 females and 188 males reared at several temperatures gave virtually perfectly regular inverse correlation of size with temperature; thus, the mean length for thirty-three 28–30° females was 1.11 mm. and for forty-two 7–10° females 1.62 mm. Apparently copepods of this species reared at about 8° temperature are generally about 50% longer than those grown at a temperature 20° higher (See figures 1 & 2). Were the proportions in dimensions the same at the different temperatures, the high-temperature copepods would have only about $\frac{1}{3}$ the volume of the low-temperature copepods. We shall see, however, that the proportions are not the same at all temperatures.

Rate of Development

Duration of the period of development was directly dependent upon temperature. Nauplii placed at 28.1° came to maturity in 6 or 7 days, but brother and sister nauplii of the same age, required four, and very often as much as five weeks to mature at 7.7°. The former period corresponds to that reported by Coker (1934a), 6–7 days, but the period of development for low-temperature copepods was notably shorter in our experiments than in his (about 50 days).

Mortality

The relative numbers of animals that came to maturity in the two groups were very different. Of 301 nauplii placed at 28.1°, 146 (48.5%) reached maturity, but of 306 at 7.7° only 24 (7.84%) attained maturity.

Form

Length of furca relative to length of body

Length of furca relative to length of body exclusive of furca, $\frac{FL}{BL}$, was compared for 23 females from the low-temperature group, and for 38 from the high-temperature group. Measurements were made under the high power of the microscope (4 mm. obj.; 10 × oc.), from preserved material. The mean ratio for the 28.1° females, 0.110 (range 0.086–0.138), was slightly lower than the mean for the 7.7° females, 0.126 (range 0.114–0.147). For 12 males reared at 28.1° this ratio ranged from 0.091 to 0.114; the only male that came to maturity at 7.7° had a $\frac{FL}{BL}$ ratio of 0.134. The data, as far as they go, suggest that length of furca relative to length of body is slightly lower for high-temperature than for low-temperature copepods, as Coker suggested, but again the evidence is inadequate for positive conclusion.

Proportions of Furca

Width of ramus was measured halfway of its length, and length measured on the outer side from the base of the ramus to the base of the spine on the outer margin (fig. 3). The mean ratio for 28.1° females (38 examples), 0.284 ± 0.0125 was about $\frac{1}{3}$ again greater than the mean for 7.7° females (23 examples), 0.217

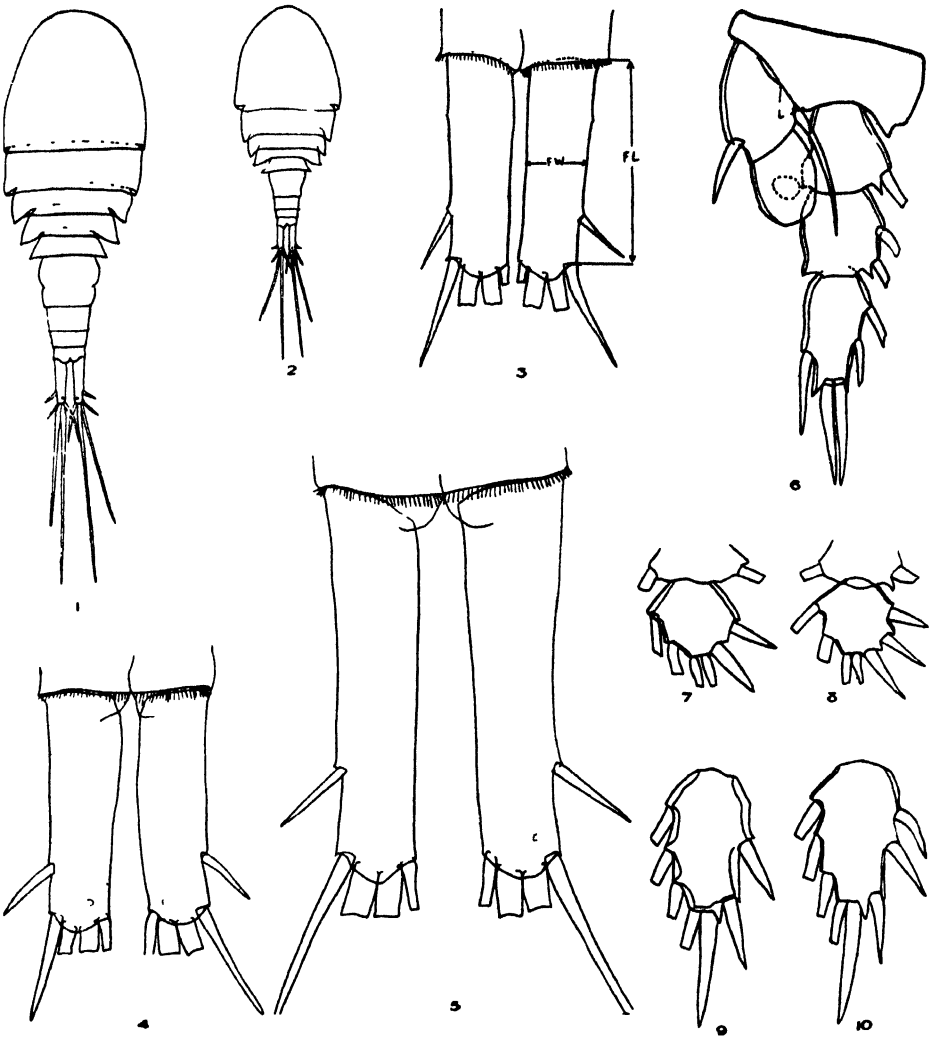

FIGURE 1. Female *C. vernalis* reared at 7.7°C. Body length, 1.632 mm.; cam. luc.

FIGURE 2. Female *C. vernalis* reared at 28.1°C. Body length, 0.922 mm.; cam. luc.

FIGURE 3. Furca of 28.1° female. Illustration of method employed in measuring animals for proportion of furcal width relative to furcal length. FL (furcal length) measured on the outer side from the base of the ramus to the base of the spine on the outer margin; FW (furcal width) taken half-way of the length of the ramus.

FIGURE 4. Furca of mature 28.1° female: $\frac{FW}{FL} - 0.345$ (extreme). Rami of high-temperature animals are wider, relative to length, than those reared at low temperatures.

FIGURE 5. Furca of mature 7.7° female: $\frac{FW}{FL} - 0.196$ (extreme)

FIGURE 6. Fourth foot of 7.7° female, showing deformed exopod: cam. luc. No terminal segment and only a portion of the second segment present.

FIGURES 7–8. Terminal segments of exopods of first feet of two 28.1° animals, illustrating variation in spine formula. Number 7 has 2 spines, 4 setae; No. 8, 3 spines, 4 setae. (cam. luc.)

FIGURES 9–10. Terminal segments of exopods of fourth swimming feet of two 28.1° copepods. No. 9 with 3 spines, 4 setae; No. 10 with 4 spines, 4 setae.

± 0.018 (cf. figs. 4 & 5). These ratios are remarkably similar to those obtained from the European *C. vernalis* by Coker (1934a), who reported 0.266 as a mean ratio for 28–30° females, and 0.195 as a mean for 7–10° females. The mean ratio for twelve 28.1° males, 0.298, and the ratio for the one 7.7° male, 0.207, also correspond surprisingly closely with Coker's record of 0.311 for 28–30° males and 0.218 for 7–10° males.

Additional data accumulated by Coker and Sheppard in 1936 (manuscript notes), were made available to the present author, and the records have been summarized as follows: The mean ratio for twenty-four 28° females, 0.323 ± 0.013, was more than ⅓ greater than the mean for twenty-one 10.2° females, 0.225 ± 0.021; for twenty-four 3.9° females, however, the mean was 0.256 ± 0.022, suggesting a tendency toward stouter furca at extremely low temperatures. A repetition of this condition occurred in males, although to a lesser extent: the mean for nine 28° males was 0.328 ± 0.013; for twelve 10.2° males, 0.241 ± 0.018; and for six 3.9° males, 0.253 ± 0.014. The results in any one of the several experiments have little statistical significance, but the general conformity of results in several series of experiments with measurements on 150 females and 60 males of different origins leaves little doubt as to the rule of direct correlation of the ratio of width to length of furcal rami $\frac{FW}{FL}$ for *C. vernalis* within the range of about 7° to 30°C., except as there may be a reversal of correlation at extreme low temperatures, lower than that at which they can readily be bred.

Spine Formula

"Spine formula" refers to the number of spines on the terminal segments of the exopods of the first to fourth swimming feet. Within the species there are known two regular formulas: $\frac{2\text{-}3\text{-}3\text{-}3}{2\text{-}3\text{-}3\text{-}3}$ and $\frac{3\text{-}4\text{-}4\text{-}4}{3\text{-}4\text{-}4\text{-}4}$ (Coker, 1934a, 1934b), once presumed to be chief justification for specific distinctions between *C. vernalis* (*parcus* in America) and *C. robustus* (*americanus* in America). The formula $\frac{3\text{-}4\text{-}4\text{-}4}{3\text{-}4\text{-}4\text{-}4}$ means that an animal has three spines on the terminal segment of the exopod of each of the first swimming feet and 4 on the terminal segments of the exopods on each of the second, third and fourth swimming feet. For economy in words the swimming feet are hereafter referred to as P1, P2, etc., and the "terminal segment of the exopod" as "TSEx."

Of 22 low-temperature animals, 18 had the regular spine formula—$\frac{3\text{-}4\text{-}4\text{-}4}{3\text{-}4\text{-}4\text{-}4}$. There were just four exceptions, one being a manifest deformity (lack of terminal segment) of exopod on P4 (Fig. 6), and another an approximate deformity, there being only 2 spines on P3TSEx, a number (for P3) not known to be characteristic of any copepod of the group. There were, then, only 2 of the remaining 18 showing deviations within the normal range—3 instead of 4 spines on P2TSEx and P4TSEx, respectively (on one side).

In the high-temperature animals, on the other hand, there was a strikingly wide variation in spine formula. Out of 40 animals examined, not a single one had either the regular 3–4–4–4 or the 2–3–3–3 formula. Thirty different combinations

occurred, out of a possible 256, exclusive of "abnormal" numbers. The greatest number with the same formula was 4 having $\frac{3-3-4-3}{2-3-4-4}$. P2TSEx showed the greatest amount of deviation from the formula with 53 (out of a possible 80) cases of 3 spines; P4TSEx ranked a close second with 46 cases of 3 spines; P3TSEx showed the least amount of deviation, with only 11 cases of 3 spines and one of 2 spines. If all the 22 animals in the low-temperature group had had the higher spine formula (3–4–4–4), the spines on the terminal segments of exopods would have totaled 660; actually there were 552 (98.8%); in the high-temperature group, with a possible 1200, the actual number was 1052 (87.7%).

In the experiments conducted by Coker and Sheppard, three temperature levels were used—3.9°, 10.2°, and 28°C. Of 22 females reared at 3.9°, 19 had the regular 3–4–4–4 formula and 3 had some irregularity; in 21 females of the 10.2° group, 16 had the 3–4–4–4 formula and 5 had irregular formulas; and of 23 females in the 28° group, 15 had the 2–3–3–3 formula, 4 the 3–4–4–4, and 4 were irregular. Of six 3.9° males, three had the 3–4–4–4 formula, 3 were irregular; of twelve 10.2° males, 7 had the 3–4–4–4 formula, 5 were irregular; and of nine 28° males, 2 had the 2–3–3–3 formula, 1 the 3–4–4–4 formula and 6 intermediate formulas. This record differs from mine in that virtually $\frac{2}{3}$ of the 28° females show a marked degree of regularity in the recurrence of the 2–3–3–3 formula: $\frac{1}{6}$ of them have the formula typical of low-degree animals, and only $\frac{1}{6}$ show irregularities of formula. I found no case of either the 2–3 or the 3–4 formula in my 28.1° animals. On the other hand, the occurrence in their low-temperature (3.9° and 10.2°) females of the 3–4–4–4 formula in 35 out of 43 cases is in line with my results. Coker and Sheppard's data are similar to the present author's in suggesting the tendency toward reduction in number of spines at a higher temperature.

If we combine Sheppard's records with mine, including as "low-temperature" copepods all reared at 10° or below (there were no significant differences noted between 10° and 4° copepods) and lumping males and females, we find that 72 copepods reared at 28° had 1,840 spines on the terminal segments of the exopods, or an average of 25.6 (instead of 30 for the 3–4–4–4 formula, both sides, or 22 for the 2–3–3–3 formula) and 82 copepods reared at 10° or lower had 2,392 spines, or an average of 29.17. The low-temperature copepods closely approximate the higher formula and the high-temperature copepods approach more nearly the lower formula, as a rule, and actually have it in many cases.

It might be surmised that the greater amount of deviation in the 28.1° copepods was a reflection of unfavorable living conditions associated with a high temperature. As mentioned in a preceding paragraph, however, about $\frac{1}{2}$ of the 28.1° animals lived, whereas only about $\frac{1}{15}$ of the low-temperature animals lived; the more regular spine formula and the higher number of spines occurred on animals reared where there was greatest mortality and where also the only two cases of deformity occurred. The irregularity is therefore not to be assumed as attributable to unfavorable conditions at the higher temperatures.

The spine formula in the species *C. vernalis* Fischer is known to be remarkably

variable. The following paragraphs are quoted from manuscript notes by R. E. Coker.

"As far as our experience goes, *C. vernalis* is exceptional among cyclopoid copepods in the diversity of spine formulas, regular and irregular, that it displays. Even in copepods, such as those generally identified as *C. serrulatus*, which show a high degree of diversity of form in other respects, deviations from the characteristic spine formula have not been encountered commonly in nature or in cultures. Possibly the *strenuus* group is as variable as *vernalis*. It is not surprising that the formula should have been generally taken as a specific character.

"With respect to *vernalis*, one of our former students (L. L. Hill) made a good many observations of spination.

"A collection of 30 copepods from a small pool near Chapel Hill, N. C., October 3, 1927, included 26 with the 2–3–3–3 formula and 4 with not more than 2 supernumerary spines. A culture from this collection yielded, of 33 examined, only three with the 2–3–3–3 formula; the remainder had supernumerary spines, and 16 displayed the complete 3–4–4–4 formula. Another culture derived from a pair, each of which was known to have the regular 2–3–3–3 formula, yielded 14 with the parental formula and 6 with excess spines, including 3 which lacked only 2 spines of having the higher formula. The significance of temperature was not then suspected and the thermometer, unfortunately, was not used.

"Examination of 26 copepods taken from the same pool in March, and therefore presumably winter-bred, revealed only one with the 2–3–3–3 formula, 23 with the 3–4–4–4 formula and two with irregularities. The population of the pool had changed from a predominantly 2–3–3–3 condition in the fall to a 3–4–4–4 condition in the spring. Of course, it is not positively known that the spring copepods with higher formulae were descendants of the fall copepods with the lower formula.

"Cross-mating yielded 5 with the low formula, 6 with the high formula and 28 with intermediate numbers. A second generation gave the same groups in the proportions 6–3–9.

"From a lake in New York State where the sub-surface temperature, frequently recorded, was never above 15°C., 50 copepods were examined and all but 2, each lacking one spine, had the regular high formula. Fifty offspring from one pair of these mated in the laboratory included only 14 with the full 3–4–4–4 formula; 35 with more or less reduction in spination, giving 23 different patterns; and one with the 2–3–3–3 formula. These copepods, closely related to *vernalis* and similarly variable as to spination, are now identified as *brevispinosus*—Marsh ("variety," subspecies or species?).

"These observations do not eliminate the possibility that there are different genetic bases for the two formulas; they do show that the spine formula in *vernalis* and most nearly related species or subspecies is neither a dependable taxonomic character nor adapted to genetic experimentation without greater knowledge of the influence of environment upon the pattern of spination.

Obviously they suggest that copepods of this group bred in cold waters have more spines than those bred in warm waters."

CONCLUSIONS

1. Animals reared at 28.1° not only reach maturity sooner than those reared at 7.7°C. but are much smaller. The low-temperature copepods are nearly 50% longer, as previously reported by Coker.

2. There was a higher mortality rate in the low-temperature group: about ½ of the 28.1° animals, but only about $\frac{1}{18}$ of the 7.7° animals survived to maturity.

3. Mean ratio of length of furca to length of body for the 28.1° animals was slightly lower than the corresponding mean for copepods reared at 7.7°.

4. In 3 sets of experiments with different lines, conducted by Coker (1933), Coker and Sheppard (not hitherto published) and the present writer, the furcal rami of 28° animals were found to be wider, relative to length, than are those of copepods reared at a temperature about 20° lower; the experiments involved measurements upon 210 copepods.

5. The spine formula of the low-temperature group, was 3–4–4–4, with only four exceptions one of which was a deformity. The spine formula of the high-temperature animals was variable, but with a marked tendency toward reduction in number of spines. None of the copepods of our experiments gave precisely the lower regular formula supposed to occur within the species, although such copepods did occur in the experiments of others cited.

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NOTES ON RARE HYDNUMS

By W. C. Coker

Plates 13–16 and 4 Text Figures

Hydnellum geogenium (Fr.) Banker. Mycologia **5**: 204, 1913.

Plate 13 and Text Figure 1

Gregarious in large numbers, often confluent and complicated; individual caps up to 6.5 cm. broad, fused clumps up to 10 cm., strongly depressed with margin elevated or spreading; surface inherently fibrous with low radiating ridges and slightly scrobiculate center, zonate; color when soaked deep wood brown, margin abruptly bright sulphur yellow for several mm.; when dry a paler wood brown with a distinct olive yellow tint, the margin yellow, the younger margins remaining bright sulphur yellow. Flesh yellow-brown, homogeneous, up to 3 mm. thick except in center, tough, very pliable, not breaking when bent on self, marginal region brighter yellow; tasteless and practically odorless when fresh but on drying becoming somewhat aromatic with a faint odor of fenugreek.

Spines up to 4 mm. long, crowded, strongly decurrent to substratum, bright sulphur yellow at margin and remaining strikingly yellow for some time, then brownish but still with yellow tips.

Stem comparatively slender, covered with spines and rather abruptly fading into the cap, about 1–2 cm. long above the large abruptly swollen amorphous mass in the substratum which shows on the surface some of the bright sulphur yellow mycelium; flesh homogeneous except for soft swollen mass below, blackish and with or without streaks of yellow.

Spores (of No. 12559) fawn colored, subglobose or elongated, irregularly warted, 3.5–4.2 x 4–5.2μ.

The fresh cap margin turns blackish when rubbed. In fully mature spines the tips are strongly yellow, the sides fawn colored. In the dry state the mycelium remains sulphur yellow. The bright yellow color of cap margin, spines and mycelium makes this the most spectacular of all the stipitate Hydnums. There is nothing else at all like it.

Our plants are the same as those collected by Peck at South Ballston, N. Y., and not otherwise reported in America (Rept. N. Y. St. Mus. **39**: 43. 1886). Through the kindness of Dr. Homer D. House, we have been able to compare our plants directly with Peck's material and find them identical. Peck's reference of his plants to Fries's species, if we take only the evidence from Fries's published description and plate, is not convincing, but from Banker's observations (Mycologia **5**: 204. 1913) we feel it necessary to adopt Fries's name, as he says that Fries's material and Peck's are the same. Fries's plate (Icon. Selectae, pl. 8) has the flesh and single stem entirely too thick and the highly complicated caps from one stem are not found in our plants.

This is another plant that does not agree satisfactorily with any accepted genus. The homogeneous context is a character of *Sarcodon*, but since it is pliable and not fleshy Banker was led to place it in *Hydnellum*. For other

anomalous species, not nicely taken care of in any genus, see the key in this Journal **55**: 381, 1939.

Georgia. Rabun County. No. 12559. In deep humus on bank of Clear Creek just inside the Georgia line at first falls on the way to Bascom Caves, Aug. 12, 1941. No. 12694. Same spot as above, Aug. 22, 1941.

Hydnellum floriforme (Schaeff.) Banker.

Plate 14

Illustrations of this species fail to give a correct idea of its appearance when most amply developed. Nearly all figures show a thick cap, fading gradually into a plug-like stem. Our figures in this Journal **34**: pls. 19 and 20, show moder-

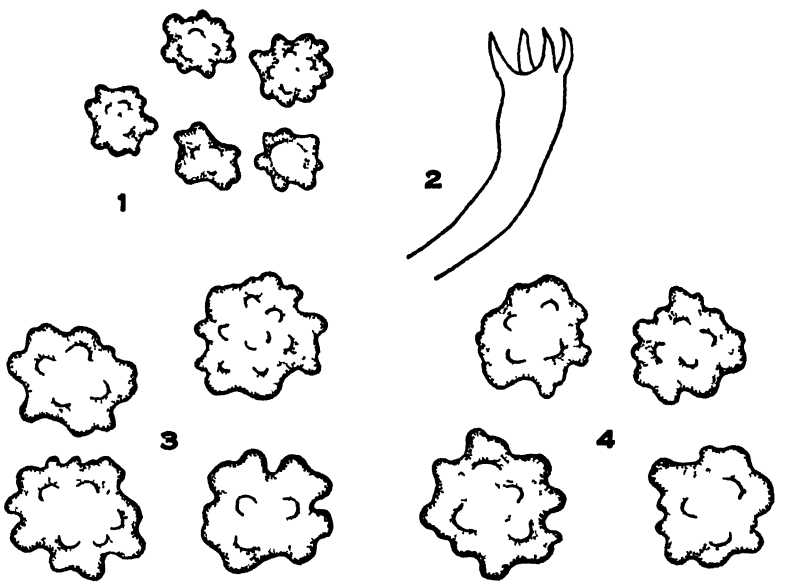


FIG 1 Spores of *Hydnellum geogenium* No 12559
FIGS 2, 3 Basidium and spores of *Sarcodon Blackfordae* No 12712
FIG 4 Spores of *Sarcodon Blackfordae* Type
Fig 2 × 1080; others × 2160

ately expanded caps, but we think it worth while to reproduce a plate showing the full development of plants in a good colony found by us in northern Georgia. We include below a rather brief description of this very handsome form.

Caps complicated and multiple on a single distinct and rooting stem, up to 12 cm. wide, rarely simple and then excentric or flabelliform, ochraceous orange, older areas brownish tawny, about russet (Ridg.); surface dull, velvety, taking the imprint of a finger when fresh, more or less zonate. Flesh duplex, outer layer soft, concolorous with surface, up to 1.5 mm. thick; lower layer firmer and browner or nearly concolorous, up to 3.5 mm. thick except near stem.

Spines up to 3.5 mm. long, crowded, ochraceous tawny when mature, darker with age, more or less decurrent but usually with a very obvious limit, due to the strong contrast in color between the brown spines and the orange stem.

Stem very distinct, simple, deeply rooted in leafy humus, up to 7.5 cm. long and 2 cm. thick, covered throughout with a spongy layer colored like the cap, inner flesh harder and concolorous with the harder cap layer.

Spores (of No. 11916) light fawn colored, about vinaceous buff (Ridg.), subglobose, distinctly warted, 3.7–4 x 4–5μ.

The flesh is tough and arid even when young, with no taste or odor except mildly fungoid, but the odor when dry, while slight, is distinctly that of machine oil.

Georgia. Rabun County. No. 11916. In rich ravine on Big Creek by Walhalla road, Aug. 22, 1940. No. 12476. In same spot as above, Aug. 1, 1941.

Sarcodon Blackfordae (Peck) Banker

Plate 15 and Text Figures 2–4

Cap 2–7.4 cm. wide, convex or gibbous-concave, dull, dry, finely felted, smooth or with a few pits or ridges; color in youth whitish fawn-drab, *black* when rubbed and in rainy weather, some black all over, older plants dark smoky buff towards margin, blackish brown in center; margin membranous, strongly incurved in youth. Flesh up to 8 mm. thick near stem, concolorous in youth but soon brown to blackish; taste bitterish, odor none.

Spines very long and stout, up to 1.6 cm., 1+ mm. thick at base, not at all crowded, quite free from stem and not shortened there, color grayish buff at first, then a fine deep brown with *buffy* tips; spines easily falling off and leaving obvious circular scars on the cap.

Stem about 5–8 cm. (rarely 10 cm.) long, 7–9 mm. thick, nearly equal or tapering upward, surface lightly fibrous, nearly smooth, color of cap but soon stained blackish below; solid at first with center stuffed, later hollow; base usually blunt, not greenish or bluish.

Spores (of No. 12712) dark buffy brown with tint of fawn, strongly warted, subglobose, 6–8.5μ. Basidia 7.4–9.5μ thick, 4-spored.

Our plants are identical with the type collection in Albany, kindly sent us by Dr. House. The types were found in Massachusetts by Mrs. E. B. Blackford in 1904, and our plants are the first collected since then.

North Carolina. Transylvania County. No. 12712. In thick leaves and humus in damp shaded place under Rhododendron and Kalmia by trail along Horsepasture River below the falls, Aug. 28, 1941. No. 12752. Same spot as above, Sept. 3, 1941. (Louise V. Coker, Coll.)

Phellodon Hesleri Coker

Plate 16

Since describing *Phellodon Hesleri* in this Journal **55**: 378, 1939, we have found excellent examples of it and publish herewith two photographs which bring out certain features not emphasized in the original description. Though somewhat obscured at times, there is a strong tendency for the thick spongy covering of the stem to end very abruptly, even concavely above, as shown in the upper left figure. Also in fully mature plants there is an unusually sharp contrast between the broad whitish margin and the much darker central region of the cap surface.

Our recent collections also show that the stem may be more pointed than blunt and that the harder stem flesh may be distinctly zoned and in the lower part with reddish areas.

North Carolina. Transylvania County. No 12755. Deciduous woods by trail along Horsepasture River, Sept. 3, 1941.
Georgia. Rabun County. No. 12629. In a bed of Galax by trail up Rabun Bald Mt., Aug. 20, 1941.

UNIVERSITY OF NORTH CAROLINA,
CHAPEL HILL, N. C.

HYDNELLUM GEOGENIUM. No 12559. Nat. Size

HYDNELLUM FLORIFORME No 11916 UPPER FIGURE × $\frac{2}{3}$, LOWER SLIGHTLY REDUCED

ARCODON BLACKFORDAE No 12732 (ABOVE), No 12712 (BELOW) Nat Size

PLATE 16

PHELLODON HESLERI No 12629 (ABOVE, SLIGHTLY REDUCED), No 12755 (BELOW, Nat Size)

A NEW ROZELLA OF THE POLYSPORANGIATE SERIES

By Leland Shanor[1]

Plate 17

The genus *Rozella* was established by Cornu (1872) to include four species of endophytic parasites of the aquatic Phycomycetes whose mature sporangia completely filled that portion of the host hyphae which they occupied. Three of these four species described by Cornu produced sporangia singly and caused some hypertrophy while the fourth formed sporangia in a linear series within the host hyphae and caused only slight hyphal enlargement. Cornu recognized that these four species represented two distinct groups within the genus. Normal zoospores in all species in which they were observed by Cornu possessed but a single posterior flagellum, but abnormal biflagellate zoospores were also seen in some cases.

Ten years after Cornu's paper was published, Fischer (1882) found and carefully studied a parasite of *Saprolegnia* which he mistook for *Rozella septigena* Cornu, and described a similar, new species on *Achlya* which he named *Rozella simulans*. Fischer's studies on these two species led him to emphasize Cornu's conclusion that there were two distinct groups within the genus. Later Fischer (1892) became convinced that these two groups of species represented distinct genera, so he proposed the name *Pleolpidium* to embrace the first three species described by Cornu. Fischer retained the name *Rozella* for what he erroneously thought was the same fungus as *R. septigena*, the atypical species described last by Cornu, and for the parasite which he had previously discussed under the binomial *Rozella simulans*. The normal zoospores of the fungi studied by Fischer were described and figured as biflagellate and, largely because Cornu had failed to observe the second flagellum on zoospores of species of *Olpidiopsis* described at the same time, most later authors have followed Fischer in considering his interpretation of *Rozella septigena* and *R. simulans* as representing the true characteristics of the genus. The zoospores of the species placed in *Pleolpidium* were accepted as being uniflagellate while those of *Rozella* were thought by Fischer and those who followed him to possess two flagella.

Sparrow (1938) has reviewed the problems relating to the confusion that has resulted from Fischer's misdetermination and has incorporated in the genus *Rozella* certain species of *Pleolpidium* that have been diagnosed since Fischer's monograph appeared in 1892 in which the latter genus was established. He had pointed out that *Pleolpidium* is invalid since the species which Fischer included here were those which embodied the conception of the type of the genus *Rozella* as originally understood and described by Cornu (1872). Sparrow (1938) recognized that the conception of generic characters for *Rozella* had to be broad and inclusive in order to accommodate *Rozella septigena* Cornu and *R. Allomycis* Foust, but he hesitated to remove these aberrant species from the genus.

[1] The author wishes to thank Professor J. N. Couch for helpful suggestions.

Karling (1942 a, b), has also reviewed the problems that exist in *Rozella* and came to the conclusion, reached earlier by Sparrow (1938), that the fungi with biflagellate, heterocont zoospores which were observed by Fischer (1882, 1892) must be renamed. Karling has proposed a new genus, *Rozellopsis* Karling, and has assigned Fischer's two fungi to it, along with two other species which possess similar zoospores. Karling based this new genus entirely upon description in the literature for he has not carefully studied any of the fungi placed in *Rozellopsis*.

Although the characteristics of the new fungus to be described in this paper indicate that it is a species distinct from *R. septigena* Cornu, the fact that it is a similar parasite of *Achlya* which produces uniflagellate zoospores formed in sporangia which occur in a linear series within the host hyphae lends weight to the argument that Cornu was correct in his observation of normal uniflagellate zoospores for *R. septigena* and that this is a valid species.

With the addition of this new species the polysporangiate group of *Rozella* now contains three species, *Rozella septigena* Cornu, (1872), *R. Allomycis* Foust (1937), and the present species.

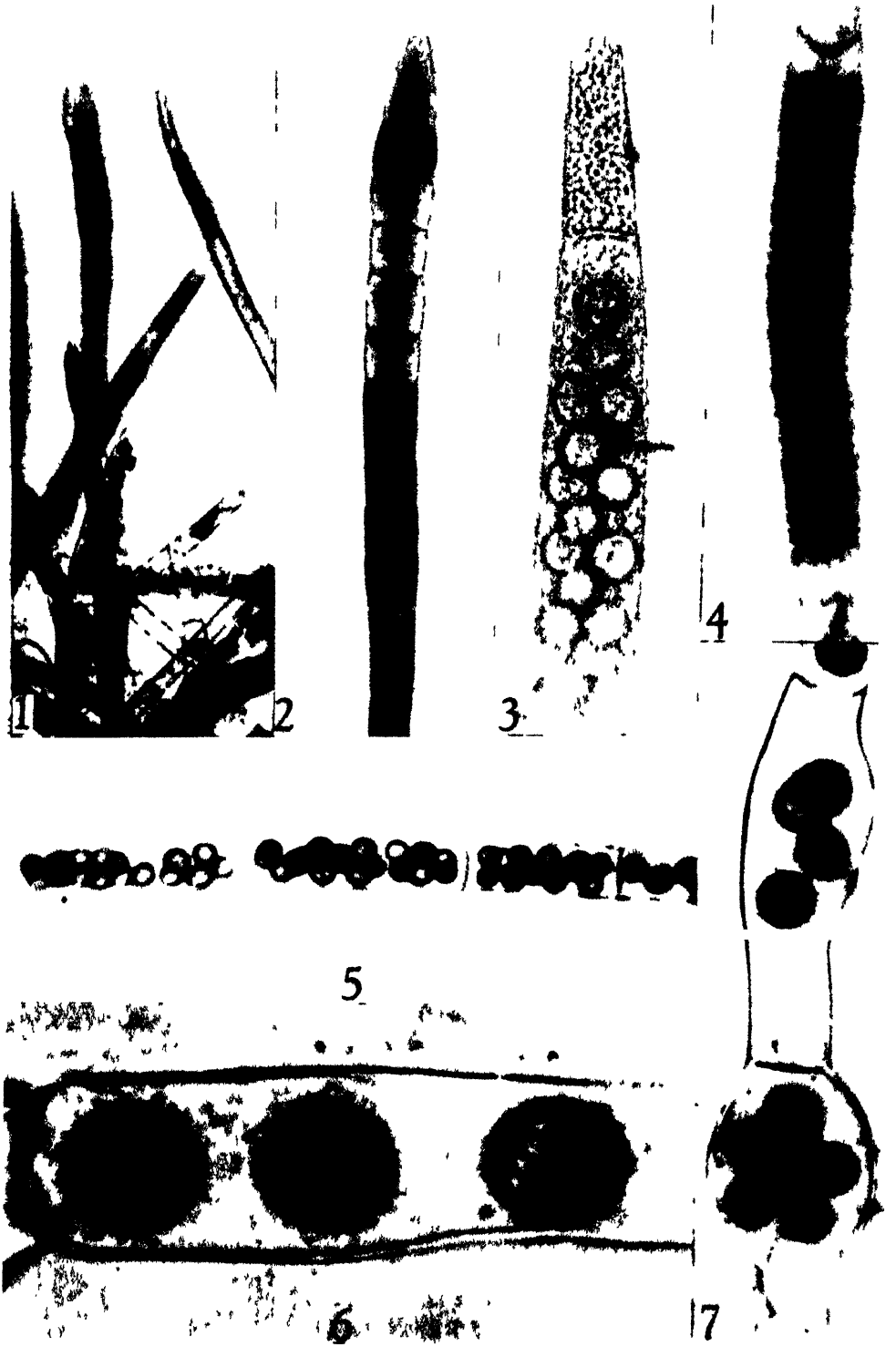
Rozella Achlyae sp. nov.

An endophytic parasite of *Achlya flagellata* causing very slight or no hypertrophy. Young plasmodium hardly distinguishable in the host protoplasm, hyaline and very nearly optically homogeneous. Sporangia formed in linear sori, cylindrical to somewhat barrel shaped, length and width depending largely upon that of host hyphae; exit papillae short, about 1.5 μ in length, rupturing following gelatinization of the tips. Zoospores swimming in a jerky and darting manner, ovoid, 2–3 x 3–4 μ with a single refractive globule, single flagellum posteriorly attached, usually 12–15 μ in length. Resting bodies produced in segments formed in host hyphae that resemble sporangial sori, each segment containing from one to many resting bodies. Resting bodies spherical to oval, 12.6–23.7 μ in diameter (not including spines), mostly 15.8–17.3 μ, usually covered with fine tenuous spines which commonly measure about 1.6–2.3 μ in length, wall of mature resting bodies thick, reddish-brown to amber brown in color. Resting spore germination follows a dormant period and is accomplished by the formation of posteriorly uniflagellate zoospores which escape through an exit papilla.

Collected by Lindsay S. Olive as a parasite of *Achlya flagellata* Coker obtained in a water sample taken from Harbison Lake, Highlands, N. C., August 1939. This plant has been collected several times previously by Professor J. N. Couch (unpublished notes) and the above collection is one of the two strains of *Rozella* sp. referred to by him (Couch, 1941, top of page 705). Karling (1942, b) has observed a parasite of *Achlya* which is similar to *R. Allomycis* Foust. This *Rozella* referred to by Karling may possibly be identical with *R. Achlyae*.

Attempts have been made to inoculate the following fungi with *Rozella Achlyae*: *Achlya imperfecta*, *A. recurva*, *A. prolifera*, *Achlya* sp. indet. (sterile), *Saprolegnia delica*, *S. mixta*, *S. ferax*, *Isoachlya unispora*, *Protoachlya paradoxa*,

PLATE 17



P. hypogyna, Thraustotheca clavata, Brevilegnia unisperma, Dictyuchus missouriensis, D. pseudodicton, Dictyuchus monosporus (sterile), and *Aphanomyces laevis*. The results of these inoculation experiments have all been unsuccessful, indicating that *Rozella Achlyae* is probably limited to a single host species.

SUMMARY

A new species of *Rozella* belonging to the polysporangiate group is described as *Rozella Achlyae*. This addition raises the total of recognized species in this group to three. The host for this parasite is *Achlya flagellata* Coker and attempts to transfer it to sixteen other species belonging to the Saprolegniaceae have been unsuccessful.

DEPARTMENT OF BOTANY,
UNIVERSITY OF ILLINOIS,
URBANA, ILLINOIS

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EXPLANATION OF PLATE 17

Rozella Achlyae

Fig. 1. Portion of an infected culture of *Achlya flagellata* Coker showing general aspect of parasitized hyphae containing both sporangia and resting spores of *Rozella Achlyae*. × 98.

Fig. 2. Tip of an infected hypha to show typical linear arrangement of developing sporangia. × 125.

Fig. 3. Portion of an infected hypha with a sporangium of the parasite at the tip and behind it a compartment containing developing resting spores. × 422.

Fig. 4. Compartment of an infected hypha containing young resting spore thalli in the dense granular host protoplasm. × 422.

Fig. 5. Portion of a typical heavily infected hypha showing several resting spore compartments, each containing several resting spores. × 188.

FIG. 6. Portion of resting spore compartment enlarged to show characteristics of the resting spores; the one to the right seen in surface view, the other two in median optical section. × 1125.

FIG. 7. Two somewhat swollen resting spore compartments, each containing several resting spores. × 422.

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PROCEEDINGS OF THE FORTY-FIRST ANNUAL MEETING OF THE NORTH CAROLINA ACADEMY OF SCIENCE

WOMAN'S COLLEGE, UNIVERSITY OF NORTH CAROLINA, GREENSBORO, NORTH CAROLINA

The forty-first annual meeting of the North Carolina Academy of Science was held at the Woman's College of the University of North Carolina at Greensboro, April 24 and 25, 1942.

The first general session was called to order by President R. E. Coker at 10:30 A. M. The presentation of papers began at once and continued until 1:00 P. M. The Biochemistry and Physiology section also met Friday morning, and the Psychology section conducted a panel session during the morning.

The reading of papers before the general session was resumed at 2:00 P. M. and continued until 3:45 P. M., when the Academy recessed to look over the demonstrations and exhibits and enjoy a bit of refreshment served by the College.

The Academy reconvened at 4:30 P. M. to transact such business as came before it. The business meeting was called to order by President Coker. The minutes of the 1941 meeting, as published in the Mitchell Journal, were approved. Reports of the various committees were then called for. These reports for the most part had been mimeographed and distributed to the members as they registered. Since the complete reports were available to the membership at the meeting (and may still be obtained by writing the Secretary) somewhat abbreviated reports are given here.

REPORT OF THE EXECUTIVE COMMITTEE

The following business was transacted by correspondence. The Committee appropriated $150 for secretarial aid to the Secretary. It also voted a bonus of $50 to the Journal of the Elisha Mitchell Scientific Society for 1941. The date for the annual meeting of the Academy was set, as was also the date for the annual meeting of the Executive Committee. The Committee authorized the appointment of a special committee to consider problems relating to industry and science. Eleven new members were elected by mail.

The regular annual meeting of the Committee (with all members but one present) was held in Durham, April 18.

An invitation to hold the 1943 meeting at Duke University was received from President R. L. Flowers. The invitation was accepted.

After a preliminary report by the Secretary-Treasurer the Committee authorized several irregularities in the published program. At the proper place the Committee will make certain recommendations which will obviate the difficulties encountered in arranging the current program. The Committee authorized the Treasurer to make a preliminary report at the annual meeting with the understanding that the final report be based upon the bank statement of June 1. The final report when certified by the auditors is to be published as a part of the 1942 Proceedings.

The Committee authorized the Secretary to secure by gift or purchase a complete file (if available) of the published Proceedings. These are to be bound and kept by the Secretary. Our earlier records are then to be transferred to some place of safety.

The Committee reports that the following have been elected to membership in the Academy.

Allen, Mr. C. M., Biology, Wake Forest.
Barton, Miss Eleanor P., Zoology, Duke.
Botany Club, W. C. U. N. C.
Brackett, Dr. Sterling, Public Health, U. N. C.
Brockman, Miss Marian, student in chemistry, Catawba.
Chow, Mr. Edward Haa-Sheng, pre-medical student, Wake Forest.
Coldwell, Miss E. Inez, Biology, W. C. U. N. C.
Conner, Mrs. Elizabeth Hatcher, Biology, High Point College.
Daniel, Dr. W. J., Psychology, U. N. C.
Darsie, Mr. J. C., Div. of Game and Inl. Fisheries, Salisbury.
Decker, Dr. J. P., Botany, Duke.
Edwards, Miss Flora White, Home Economics, W. C. U. N. C.
Endicott, Dr. Margaret M., Chemistry, W. C. U. N. C.
Evans, Dr. F. G., Zoology, Duke.
Ferguson, Dr. F. F., Biology, Norfolk College of William & Mary.
Fritz, Dr. R. L., Mathematics, Lenoir Rhyne.
Grochola, Mr. C. W., Long Island, N. Y.
Harrison, Dr. T. P., English, State College.
Heffner, Miss Madeline C., Biology, W. C. U. N. C.
James, Mr. B. H., Div. of Game and Inl. Fisheries, Robersonville.
Jordan, Mr. C. D., Zoology, Duke.
King, Dr. Willis, Dept. of Conser. & Develop., Raleigh.
Kinsman, Dr. Gladys M., Home Economics, W. C. U. N. C.
Kistler, Mr. J. W., Dept. of Conser. & Develop., Raleigh.
Knudsen, Mr. A. R., Agronomy, State College.
Kyker, Dr. G. C., Biological Chemistry, U. N. C.
Loeppert, Dr. R. H., Chemistry, State College.
Love, Miss Lila Bell, Biology, W. C. U. N. C.
McKnight, Dr. R. B., 403 N. Tryon St., Charlotte.
McLean, Mr. L. T., Horticulture, State College.
Mecklenburg Audubon Club, Charlotte.
Milam, Mr. F. M., Agronomy, State College.
Newcomer, Dr. E. H., Botany, U. N. C.
Phy-Chem Club, Durham High School.

Poole, Mr. F. G., Botany, U. N. C. (present address unknown).
Ray, Dr. Charles, Jr., Central Fibre Corp., Pisgah Forest.
Reardon, Dr. Anna J., Physics, W. C. U. N. C.
Rice, Dr. W. A., Geology, U. N. C.
Shamburger, Miss Anne, Hygiene, W. C. U. N. C.
Sigma Pi Sigma, Alpha Chapter, Davidson College.
Smith, Dr. B. E., Biology, Coker College, Hartsville, S. C.
Smith, Mrs. Susan Gower, Duke Medical School.
Spencer, Mr. C. G., Carthage.
Stainback, Mr. Raymond, Physics, U. N. C.
Stevens, Mr. R. O., Zoology, State College.
Taylor, Mr. M. H., Div. Game and Inl. Fisheries, Kinston.
Van Poole, Mr. T. B., Jr., Physics, Catawba.
Wauchope, Mr. Robert, Anthropology, U. N. C.
Webb, Dr. Bailey, State Laboratory of Hygiene, Raleigh.
Whitney, Dr. J. B., Jr., Central Fibre Corp., Pisgah Forest.
Zoology Field Club, W. C. U. N. C.

The Secretary was authorized to prepare a mimeographed list of the membership and distribute the same to departmental heads and other interested members.

The Committee authorized the Treasurer to purchase war bonds to the extent of $300.00 for our savings account. These are twelve year bonds with an initial expenditure of about $225.00. A balance of nearly $200.00 would be left in the savings account.

The Academy appointed Dr. E. W. Gudger to represent the Academy at the celebration of the 75th Anniversary of the Torrey Botanical Club in June.

The Committee makes the following recommendations:

1. That each member shall be limited to one paper on the general program at any one annual meeting.

2. That the recipient of the Poteat Award be announced as a part of the Proceedings.

3. That a committee be set up to consider the criteria for life membership.

4. That the following be elected to life membership in the Academy: S. C. Bruner and J. D. Ives.

5. That the following amendment to the Constitution be adopted:

Article II, section 1, to read:

(a) Members. Any person actively interested in science or the promotion of science, may, upon the payment of the annual dues of $2 and upon nomination by two members, be elected a member of the Academy by a majority vote of the Executive Committee, and shall be entitled to all the privileges of the Academy.

(b) Junior Members. Any person of high school grade who has shown unusual aptitude and accomplishment in some field of science may be elected to Junior Membership by a majority vote of the Executive Committee. Such membership is for the current year and there are no dues.

(c) Life Members. Members may be elected to life membership by the Academy upon nomination by the Executive Committee. Such election

relieves the member from further payment of dues, but does not invalidate his right to present papers, vote, and hold office.

(d) Sustaining Members. Institutions, organizations, or industrial concerns may be elected as sustaining members by the Executive Committee upon the payment of a nominal fee. All funds collected from this source are to be placed at the disposition of the Research Grants Committee.

(e) Patrons. Any person contributing a sum of $10 or more annually to the Academy may be elected by the Executive Committee as a Patron. All funds collected from this source shall be at the disposal of the Research Grants Committee.

(f) Affiliates. Active organizations having scientific objectives may upon application and the payment of annual dues ($2.00) be elected by the Executive Committee as affiliates. The affiliation may be renewed from year to year by action of the Executive Committee. Affiliated organizations shall be entitled to receive the Journal or some substitute and such notices as are issued to the members of the Academy. Each such affiliate shall be entitled to elect a delegate to the Academy who shall have the right to vote.

Article II, section 2, to be deleted Refers to initiation fees.

Article II, section 3, to be renumbered 2 and the word "old" deleted.

Article II, section 4, to be deleted Refers to patrons which are now covered in Section 1.

6. That the High School projects be continued

7. That the Academy set up a special Committee to codify the Constitution and By-laws and perform related functions

The recommendations were each adopted separately and then the report was adopted as a whole.

TREASURER'S REPORT

Expenditures

Seeman Printery (Stationery*)	$49 22
Stamps	6 00
Nell Starling (Sec Asst)	20 00
Duke University (Lantern slide boxes)	8 49
Nell Starling (Sec Asst)	15 00
Seeman Printery (File cards)	20 00
Mitchell Journal (Bonus)	50 00
Nell Starling (Sec Asst)	25 00
Intang Tax	23
Audubon Soc. (Lantern slides)	50 00
Seeman Printery (Stationery)	26 60
Bank charge	1 00
Waverly Press (Reprints of Proceedings)	8 70
Nell Starling (Sec Asst)	27 50
Naomi Mann (Sec Asst)	10 00
Rubber check	2 00
Ernest Hardwicke (H. S Award)	20 00

*Includes stamped envelopes

Marguerite Lyon (Sec Asst)	8 50
Stamps	3 00
Inez Wetmore (Sec Asst)	6 00
Seeman Printery (Announcements)	16 25
Bert Cunningham (Sec Comm)	12 00
Jour E M S S (1942)	300 00
Bert Cunningham (Exp at meeting)	8 30
Bert Cunningham (Sec Comm)	25 00
Bert Cunningham (Refund dues)	2 00
Naomi Mann (Sec Asst)	4 40
Zoology Department (Stencils, paper)	15 00
Bert Cunningham (Sec Comm)	20 00
Rose Agency	1 75
Naomi Mann (Sec Asst)	8 80
Bank charge	1 00
Seeman Printery (Programs)	37 50
Nell Starling (Sec Asst)	14 00
Nell Starling (Asst at meeting)	5 00
Nell Starling (Sec Asst)	20 00
Total Expenditures	$848 24

The above bills were approved by President Coker who acted upon authorization of the of the Academy and they were then submitted along with other records to the Auditing Committee

Receipts 1941-42

Bank Balance 6/1/41	$254 77
Back dues	18 00
Current dues	649 00
1943 dues	4 00
Mrs E B Clarkson (slides)	50 00
Chemists (for program)	5 00
	$980 77

Summary of Accounts

Receipts	
Bal in bank 6/1/41	$254 77
Receipts	726 00
Total	$980 77
Expenditure	
Total	$848 24
Bank balance 6/2/42	132 53
	$980 77

Savings Account

Balance 1/1/41	$416 81
Interest 1/1/41 to 1/1/42	7 31
	$424 12

AUDITORS' REPORT

On this 17th day of June, 1942, we have examined this account and found it to be correct.

JOHN B. DERIEUX
J. G. BOOMHOUR
DONALD B. ANDERSON, *Chairman*

REPORT OF THE RESEARCH GRANTS COMMITTEE

A majority of the Research Grants Committee has recommended that the A. A. A. S. fund be divided between E. E. Brown, Davidson College, and H. W. Jensen, Asheville Farm School. The studies which they wish to prosecute are as follows:

E. E. Brown—"Life history studies of the lizard, *Sceloporus u. undulatus* and the water snake, *Natrix septemvittata*." The fund is to be used for cages and other technical accessories.

H. W. Jensen—"Cytology of the genus *Ilex* with emphasis on the question of unisexuality and its influence on the meiotic process." The fund is to be used for cytological reagents and equipment.

O. C. BRADBURY
H. D. CROCKFORD
C. F. KORSTIAN
J. N. COUCH
B. W. WELLS, *Chairman*

REPORT OF THE REPRESENTATIVE ON THE COUNCIL OF THE A. A. A. S.

Your representative attended all the sessions of the Council. Since a complete report of the Council actions has been published, no further report seems necessary.

BERT CUNNINGHAM, *Representative*

REPORT OF THE COMMITTEE ON CONSERVATION

Last year your committee made a progress report or, more correctly, a report on lack of progress on two projects in which this Academy had shown its interest, namely, the Primeval Forest near Highlands and the Dismal Swamp. The former was referred to a special committee which has already reported (or is to make its report at this meeting).

The acquisition and protection of the Dismal Swamp seems necessarily postponed until the war is over, that is, unless it can be conclusively shown that a fire burning in this swamp, so close to the Norfolk area, would seriously disrupt Army, Navy and industrial activities by covering the region with a pall of smoke which might, in a dry season, last for weeks. A suggestion has already been made to the Government that steps to prevent such a catastrophe might well be considered a necessary part of our war effort.

Since no specific problems have been presented to our committee during the past year, it is deemed timely to call the attention of the Academy to the war time condition of our forest resources with the practical objective of extending so far as possible the benefits of productive timberlands into the post war period, when they will be so much needed. Not only is timber essential to the rebuilding of a civilization but such beneficient influence as erosion and flood control, the restoration of wild life, the use for recreation and aesthetic enjoyment must serve the world to the fullest extent possible. We are, therefore, presenting the following facts:

North Carolina has an approximate forest area of seventeen million acres, outside of the nearly one and a half million acres Federally owned and protected lands. Of this, some one-third is hardwoods and two-thirds pine, according to the Timber Survey made by the U. S. Forest Service in 1937. Even then the rate of growth was slightly less than the rate at which the timber was being used. With the increased use for war purposes it is estimated that this year the cut will exceed the growth by 147 million board feet; while the increased risk from fire due to the war, especially in our Coastal Plain Region, makes us all very apprehensive as to the probable post war conditions of our forests.

This matter is not presented with the thought that we should urge the saving of needed timber; rather we must cut and use every stick that can in any way contribute to the prosecution of this war, but this can and should be done without leaving the land unproductive. The object of this report is to call attention to the vital need of taking every precaution to conserve the resources from which future timber supplies must spring, i.e., the soil, the seed and the young growth of the forests that we are now cutting. If these fundamental assets are retained with as little injury to their reproductive capacity as possible the needs of the reconstruction period can be met; but if no care is given and everything salable is cut from the land without thought of any future crop and fire follows, as is especially likely in war time, then the next generation will be defrauded of its rightful heritage. To reconstruct the state or the world without forest resources would be a task too hard and dreary for us to be willing to leave to our children. Some of you will recall that one of the first contributions this country made in 1917 to the World War was the 10th Regiment of Engineers (Forestry) to be supplemented a little later by the 20th Engineers. These two regiments, officered and manned by American and Canadian foresters and lumber jacks, were sent to England and France where they operated in the woods behind the armies, getting out timbers, trees and lumber required for military purposes. While the main object was to get out timber as quickly and economically as possible, forestry principles continued to govern even the cutting of timber for war purposes. We need to be as careful of our forests as Europe was 25 years ago.

The three most important features of a war time forestry program are: (1) Fire control that will really protect the forests from fire, (2) cutting practices that will assure regeneration and succeeding forest crops, (3) close utilization that will waste nothing that has a use.

1. This state has a fire control organization extending more or less protection to the forests of 58 counties. It has had to operate on a state appropriation of $58,000, or less than one-half cent per acre of forest land covered. This is sadly inadequate for peace time conditions but with sabotage and direct attack more than a possibility, it is laying open to destruction our immense forest wealth to have no more money than that to give even ordinary protection to seventeen million acres of private forest land. The Governor of California has just approved an appropriation of over four million dollars to protect the forest wealth of that state.

2. Our methods of cutting timber for lumber, pulpwood, cross ties, fuel wood, etc., have improved very considerably in the past few years through the educational activities of the several State and Federal agencies which have been helping the landowner, but only the surface can be touched with the limited personnel available. Cutting methods must be adapted to each particular tract and should be determined in advance by the owner, with the advice of a forester. Clear cutting without adequate provision for regeneration is only justified where the land is to be cleared for agriculture, and timber stand improvement for fuel and other farm use is often desirable for making forests more productive. Cutting to a diameter limit for saw timber should be practiced only when the limit is large enough to assure that plenty of small trees will be left. Selective cutting can with advantage be adapted to many conditions. The principle of sustained yield or the regular production of annual maximum crops of timber over a long period of years should be the objective on most lands where ownership is fairly stabilized. The present supply of men competent to advise landowners would be sadly inadequate even in peace time but under war conditions, with the necessity of laying sound plans for restoration when peace is gained, more trained men are needed. State and Federal allotments for this educational and demonstration work should be increased.

3. High prices caused by war demands will do more to bring about close utilization than anything else. Low grade trees, which in normal times are unmarketable, can now be cut at a profit and the condition of the forest greatly improved by their removal. Even such usually waste material as tree tops and laps and slabs at the saw mills will find a market for fuel with supplies of coal and fuel oil greatly restricted.

Two other features of our state forestry program—i.e., land acquisition and forest planting, though necessarily delayed, must be carefully planned for, so that when peace comes an extended public forest policy can find constructive employment for the men released by war industries and the returning defenders of our country.

C. F. KORSTIAN
W. C. COKER
J. P. GIVLER
H. J. OOSTING
J. S. HOLMES, *Chairman*

The report was adopted.

REPORT OF THE HIGH SCHOOL COMMITTEE

The Committee has carried on its usual activities, although at a lower level. Only a part of the high school district meetings were attended by a representative of the Committee, but the state meeting was attended by C. F. Dodson who made the principal address.

Our attempts to organize science fairs this year have not been very successful, since, so far only one has been reported. We know that war conditions have been responsible for the discontinuance of others, and prevented still another from being organized.

The state has been pretty thoroughly canvassed by the Science Clubs of America, sponsored by Science Service, Inc., of Washington, D. C., and it may be desirable to yield the entire field to them. They can and do offer the science clubs more than we do in return for their dues. Perhaps we could do more by offering some journal or periodical other than the Mitchell Journal (which is quite beyond their level) as a part of their membership. Possibly funds could be secured for an inexpensive inter-club publication, which would be of considerable value in consolidating the work of the clubs. If Science Service follows the plan of the American Institute, which it claims to have taken over, the Academy will soon be faced with the problem of a Junior Academy of Science. Under these circumstances a special committee should be set up to study the situation.

The Committee is happy to announce the addition of a hand-colored set of slides on the Water Birds of North Carolina, to be added to our High School Loan Series. These were provided through the generosity of Mr. and Mrs. E. O. Clarkson of Charlotte, and of the North Carolina Bird Club. The syllabus was written by Mrs. M. W. Johnson.

The lantern slides provided by the North Carolina Forestry Association are excellent and have been well received by high school audiences. The Committee wishes to express its appreciation to the donors for both sets of slides.

While our slide loans have not been proportionately as large as last year, we have reached more schools and more students. The Committee feels that this is a very helpful project and urges that those members who can provide a series of slides in some field communicate with the Committee.

The Committee is glad to report that the North Carolina Forestry Association has continued its award for a meritorious essay written by a high school student on some subject related to forestry.

The Committee recommended to the American Association for Advancement of Science, that Lucille Weathers of the Garner High School and Bobby Wood of the Durham High School be made Honorary Junior Members of the Association. The recommendation was accepted and they were duly elected.

Both the meeting for high school teachers and the one for club sponsors last year were well attended. The discussions were helpful, and both meetings proved very successful. The Committee recommends that both meetings be continued as long as the interest is maintained.

At the time of the preparation of this report the Committee has no information concerning the possible entrants for the Academy award. Whether or not there are any the Committee suggests the continuation of the project.

The Committee recommends:

(1) That a special committee be set up to study the problem of a Junior Academy of Science. The Committee to report back to the Executive Committee before April 1, 1943. Adopted.

(2) That the Academy authorize the High School Committee to solicit funds for an inter-club publication, and if such funds become available to proceed with such publication. Adopted.

(3) That the Academy authorize the Secretary of the Academy to substitute some other magazine for the Elisha Mitchell Journal for club affiliates. Adopted.

(4) That the projects now under way, including the $20 Academy Award, be continued. Adopted.

(5) That the Academy express its appreciation to Mr. and Mrs. E. O. Clarkson and the North Carolina Forestry Association for their splendid cooperation with the High School Committee. Adopted.

M. L. BRAUN
C. F. DODSON
J. H. HIGHSMITH
R. J. SLAY
J. W. WOOD
B. CUNNINGHAM, *Chairman*

The report was adopted as a whole.

REPORT OF THE LEGISLATIVE COMMITTEE

The Committee has met by correspondence and the Chairman has consulted with various persons interested in our forests, streams, conservation, and publication of results of scientific investigation in our area and state, and support for the Academy.

The Committee recommends that the following program be adopted:

(1) That the North Carolina Academy of Science is in full sympathy with the program of the North Carolina Forestry Association and recommends such legislation as may be needed to carry out this program.

(2) That the North Carolina Academy of Science recommends the passage by the Legislative body of the bills sponsored by T. S. Johnson, Chief Engineer of the Department of Conservation, and W. H. Booker, Chief Engineer of the Health Department, for the control and abatement of stream pollution.

(3) That the Academy authorize the Legislative Committee or whoever the Academy may select or appoint to request the Legislature to make available annually a sum of money for the support of the State Academy, to be used for the publication of the results of research in the state and for grants-in-aid for useful research in the state. This sum to be administrated by the Executive Committee of the Academy.

(4) That the Legislative Committee be authorized to inform the Legislature and the Budget Committee and the Advisory Budget Committee of the need for funds by the several branches of the Department of Conservation and Development which are engaged in basic research for publication of said research.

(5) That members of the Academy call attention, in writing, of the Legislative Committee to any suggestion they may have in keeping with the work of the Committee.

R. W. BOST
H. F. PRYTHERCH
B. W. WELLS
WILLARD BERRY, *Chairman*

The report was adopted.

At this point the following resolution was presented by Willard Berry and C. F. Korstian:

RESOLVED that the North Carolina Academy of Science recognizes the vital need for timber, not only in the prosecution of the war, but in reconstruction after the war and especially in the future. We, therefore, endorse the forestry program of the State Department of Conservation and Development and the North Carolina Forestry Association. The Academy pledges its support in the aid of this program. The Legislative Committee is hereby authorized to cooperate with the North Carolina Forestry Association in the attainment of this program.

The resolution was adopted.

REPORT OF THE REPRESENTATIVE TO THE ACADEMY CONFERENCE

The Academy Conference held its fifteenth annual session in the Baker Hotel in Dallas, Texas, on the afternoon of December 29, 1941. Twenty-one Academies were represented, and the A. A. A. S. was represented by four members of the Executive Committee.

In the absence of President P. D. Strausbaugh of the West Virginia Academy of Science, Vice-chairman S. W. Bilsing of the Texas Academy of Science presided at the Conference.

The formal program consisted of two papers, one by Dr. E. C. Faust (New Orleans Academy), on "A Resumé of A. A. A. S. Research Grants," and the other by Dr. J. C. Godbey (Texas Academy), on "The Organization of a Collegiate Division of the Texas Academy of Science."

The paper presented by Dr. E. C. Faust was a continuation report of a paper presented at the Columbus, Ohio, Academy Conference in 1939. In this report the following facts were brought out: First, that very little money for research is added by the respective Academies to the grants allotted them by the A. A. A. S. Second, there is very little evidence that research projects reach the publication stage except as abstracts in Academy Transactions. Third, with few notable exceptions, Secretaries have great difficulty in obtaining progress reports from Grants Committees or directly from grantees. In some instances there is definite evidence of poor record keeping in Secretaries' offices; in some instances, poor cooperation between Grants Committees and Secretaries; in many instances, utter disregard on the part of grantees for requested information.

The following recommendations were presented:

(1) A. A. A. S. Executive Committee and Academies (as local representatives of A. A. A. S.) should give serious consideration to allotment of rewards with reference to responsibility of grantees to provide annual (or semi-annual) progress reports to Research Grant Committee or Secretary of Academy.

(2) Closer cooperation is needed between Research Grant Committee and Secretary of Academy. The Committee and Secretary should have readily available up-to-date duplicate files of status of each grant from 1935.

(3) In the future Mr. Woodley's office of the A. A. A. S. should be the clearing office for all such reports to the Academy Conference.

In the discussion that followed this paper Roger C. Smith (Kansas Academy) reported that his Academy does not give the amount of the grant in a lump sum

to the grantee, but allots the grant and permits the grantee to issue bills to the Secretary of the Academy against the amount allotted to him.

It was suggested that next year a report be given in the Academy Conference concerning the best method for handling grants. Accordingly it was moved and voted that the Chairman appoint a committee to make a study and report back to the Academy Conference next year an effective means of deciding to whom grant allotments are to be made, and how to handle these allotments. An amendment was voted to the above motion that the committee try to evaluate in addition any other things pertaining to the grant situation that were not mentioned in the above motion. Subsequently Dr. G. W. Prescott (Michigan Academy) was appointed as chairman of this committee and was authorized to get information from and enlist the services of any one he may choose to help canvass the situation and submit recommendations concerning the handling of research grants.

Dr. J. C. Godbey, in his discussion of "The Organization of the Collegiate Division of the Texas Academy of Science," reported that this division was organized in 1936, but showed its largest growth during the past year.

Any science club or society in any College or University of Texas, having a membership of ten or more student members, at least five of whom are members of the Texas Academy of Science, is eligible to membership in the Collegiate Division and is called a Chapter.

Each organization is allowed one official delegate to the annual meeting for each ten members. The annual meeting is concurrent with that of the Texas Academy of Science, at which time sectional meetings for the reading of papers and reports and a business session are held.

The Texas Academy issues a charter to each Chapter on its becoming an approved member of the Division.

Each student member of a chapter who is also a member of the Texas Academy of Science pays an annual student membership fee of one dollar to the Texas Academy.

At the complimentary dinner which followed the last report, the nominating committee consisting of Doctors Lyell Thomas (Illinois Academy); Septima Smith (Alabama Academy); and E. C. L. Miller (Virginia Academy) submitted the name of G. W. Prescott (Michigan Academy) as their nominee for vice-chairman. There being no other nominations Dr. Prescott was duly elected vice-chairman for 1942.

Dr. E. C. L. Miller (Virginia Academy) reported, for the Committee on Junior Academy Relationships, that nothing was accomplished since the American Institute passed out of the picture. Dr. Miller's resignation as chairman of the committee was accepted. At this time Watson Davis of Science Service, representing the Science Service, was given permission to explain the relationships his organization proposes to maintain with the various Junior Academy organizations. After considerable discussion and by common consent a committee consisting of President S. W. Bilsing; Vice-chairman G. W. Prescott; Permanent Secretary, A. A. A. S., F. R. Moulton; and General Secretary, A. A. A. S., Otis

W. Caldwell, was authorized to assist in selecting a committee to study the Junior Academy situation. The committee thus formed consists of Lyell J. Thomas, Chairman (Illinois Academy); G. W. Prescott (Michigan Academy); William G. Camp (Maryland Academy); G. L. Cross (Oklahoma Academy); R. C. Smith (Kansas Academy); Mrs. E. Barry Walker (Commerce, Texas); H. E. Enders (Indiana Academy); E. C. L. Miller (Virginia Academy); D. B. Lawrence (Minnesota Academy), and Anna A. Schneib (Kentucky Academy).

BERT CUNNINGHAM, *Representative*

The report was adopted.

REPORT OF SPECIAL COMMITTEE ON PRIMEVAL FOREST

Your "Special Committee on The Primeval Forest," appointed in compliance with a resolution adopted at last year's business meeting, has unfortunately little progress to report.

The Ravenel Tract, known as the Primeval Forest, lies on the south side of highway No. 64, some five miles east of Highlands, N. C. It is partly surrounded by the Nantahala National Forest and therefore eligible for purchase by the Forest Service. The whole property contains some 1700 acres, but since the death of Mr. Samuel Prioleau Ravenel, the former owner, his widow has arranged with Mr. William P. Pierson of Highlands, to exempt the northern part of the tract which contains the lake, and a proposed residential development through which the highway runs.

On August 18 last, Dr. W. C. Coker and the chairman called on Mr. Pierson in Highlands and he showed us a sketch map of the property, a copy of which we later secured. A blueprint of this map, redrawn, is herewith submitted.* The owner seems to have more than one way of dividing the property for which the map shows two six-hundred acre tracts outside the reserved area. Mr. Pierson discussed with us a possible 847 acres to include the Primeval Forest and Whiteside Mountain. Mr. Pierson accompanied us to High Hampton Hotel at Cashier's Valley to see Mr. Julian Miller, of Charleston, S. C., attorney for Mrs. Ravenel, and a brother-in-law of hers. Mr. Miller stated that Mrs. Ravenel was offering 700 acres, including the Primeval Forest but excluding the lake, at $57.00 per acre. In our conversation with Mr. Miller, we stated that no money was now available for the purchase of this tract although the U. S. Forest Service was interested in its acquisition but that probably the maximum price that they could pay would not exceed $25.00 per acre. This would be based upon a careful estimate of the timber and other values. It was suggested by us that Mrs. Ravenel consent to sell to the government at the maximum price they might offer and donate such other values as she felt there were in the property for the privilege of naming the area for her husband and his family in recognition of the high service he and they had rendered in reserving this unique tract of virgin timber. Mr. Miller promised to see Mrs. Ravenel and let us know her attitude. Later we were advised through Mr. Pierson that the owner was unwilling to sell the 1200 acres, excluding the lake and proposed residential site, for $25.00 per acre, as pro-

*This map is too large to be published in the Proceedings.

posed by us. "The price of the 1200 acres at the present time (September 15), Mr. Pierson wrote, "is $60,000 cash." This absolutely unreasonable position seemed to close the door and nothing was done by your Committee through the winter. Rather recently we have heard through a connection of the family, that while no concession has yet been made in price, Mrs. Ravenel has stated that she will not sell the timber for lumbering. Under these circumstances it has not seemed necessary or advisable to call the whole committee together, but the chairman recently conferred with the other Raleigh member and we agreed to recommend the continuance of the Committee so that any favorable change in the situation might be turned to advantage. We would also like to recommend that the Ecological Society of America and the Academies of our near-by sister states be invited to lend such aid as they may be in a position to offer.

B. W. WELLS
O. C. BRADBURY
H. J. OOSTING
A. F. THIEL
LIONEL WEIL
BERT CUNNINGHAM
J. S. HOLMES, *Chairman*

The report was adopted.

Dr. Holmes then presented the following resolution:

That the Special Committee be continued and that it be instructed to solicit such interest and aid from other organizations, especially the Ecological Society of America and the Academies of Virginia and South Carolina, as they may be able and willing to give.

The resolution was adopted.

REPORT OF A SPECIAL RESOLUTIONS COMMITTEE

In the death of Charles Wardell Stiles, January 24, 1941, the world lost a most worthy contributor to the advancement of zoology, particularly in its relation to public health. He first showed that the inefficiency of people in many rural districts in the southeastern United States was due to hookworm infestations. He was responsible for founding of the Rockefeller Sanitary Commission (1909–1914), which later expanded into the International Health Board and uplifted backward peoples throughout the world. He also gave notable service as secretary of the International Commission on Zoological Nomenclature (1898–1930).

Dr. Stiles was born in Spring Valley, N. Y., May 15, 1867. He attended Wesleyan (1885–1886), College de France (1886–1887), Berlin Universitat (1887–1889), and received A. M. and Ph. D. degrees at Leipzig (1890) and M. D. in Paris (1896). He also held honorary M. S., S. C. D., LL.D., and M. D. degrees from Wesleyan, North Carolina, Richmond, and Yale.

In various capacities Dr. Stiles gave outstanding service to mankind: Zoologist, United States Bureau of Animal Industry (1891–1902); Professor Medical Zoology, Georgetown University (1892–1906); Zoologist, United States Public Health Service (1902–1910), Assistant Surgeon General (1919–1930), Medical

Director (1930); Professor of Zoology, Rollins College (1932–1938). He was Honorary Custodian of Helminthological Collections in the United States National Museum (1893–1930); Lecturer, Army Medical School (1894–1901), Johns Hopkins (1897–1937), Navy Medical School (1902–1917). In 1898–1899 he served as Agricultural and Scientific Attaché at the United States Embassy in Berlin. He was delegate to the International Scientific Congresses in Leyden (1895), Cambridge (1898), Berlin (1901), Berne (1904), Boston (1907), Budapest (1927), and Padua (1930).

Dr. Stiles was a member of the American Society of Zoologists, Fellow of the Royal Society of Tropical Medicine, Corresponding Member of the Zoological Society of London, three French societies, and various others. He was a member of the North Carolina Academy of Science from 1910 to 1941, and was elected a life member in 1932.

The North Carolina Academy of Science regrets the loss of one who has passed a long and useful life and records its pride in his notable attainments, which we all honor.

STERLING BRACKETT
ARCHIE SHAFTESBURY
A. S. PEARSE, *Chairman*

The resolution was adopted by a rising vote.

REPORT OF THE POTEAT AWARD COMMITTEE

Your committee on the Poteat Award makes the following report:

The award for 1942 shall be awarded J. P. Decker of Duke University for his paper on "The Effect of Temperature on Photosynthesis in Red and Loblolly Pines."

Your committee wishes to call attention to the difficulty of arriving at a decision because of the organization of the Academy into sections. We urge the adoption of some more feasible plan by which satisfactory decisions may be reached.

D. K. ADAMS
WILLARD BERRY
M. L. BRAUN
E. H. HALL
E. L. MACKIE
G. H. SATTERFIELD
W. L. PORTER, *Chairman*

Although this report was not given at the meeting, the Secretary was instructed to include it in the Proceedings of the Academy.

The Secretary was also instructed to publish the complete list of the recipients of this award which follows:

POTEAT AWARDS

1936. F. G. Hall (Duke), Physiological Studies at High Altitudes.
1937. J. N. Couch (U. N. C.), A Fungus that Catches Nematodes. Jour. E. M. Sci. Soc. 53: 301.

1938. H. F. Prytherch (U. S. B. F.), Life Cycle of a Sporozoan Parasite of the Oyster. Jour. Morph. 66: 39–62.

1939. F. H. McCutcheon (State College), Respiratory Mechanism in the Grasshopper. Ann. Amer. Entomol. Soc. 32: 35–55.

1940. N. F. Conant (Duke), A Case of Darling's Histoplasmosis Caused by *Histoplasma capsulatum* in a Three Months Old Infant. Jour. Bacter. 41: 563–579.

1941. Alma Whiffen (U. N. C.), The Role of Chytrids in Cellulose Decomposition. Jour. E. M. Sci. Soc. 57: 321–330.

1942. John P. Decker (Duke), The Effect of Temperature on Photosynthesis in Red and Loblolly Pines.

Report of the Committee on Nominations

In considering the important subject entrusted to your Committee on Nominations, it was found that with a present 20% membership of women, only three times had a woman been elected to any office in the forty-year life of the Academy. It was also found that most of the offices have been going to the Big Six Colleges, namely, the three of the Greater State University, with Duke, Wake Forest, and Davidson. These conditions might be looked upon as discouraging membership from the smaller colleges and institutions. However, on studying Academy activity, the disparity was not as great as appeared on the surface, that is, few women or members of the smaller colleges had maintained continuous membership, had attended regularly, or had contributed papers.

Your Committee, therefore, in presenting the following slate, desires to encourage our women members and the representatives of our small colleges to take a more active part in the meetings and affairs of the Academy.

Your Committee desires to make the following nominations:

For President: Dr. H. F. Prytherch, Director, U. S. Marine Laboratory, Beaufort, N. C.

For Vice-President: Dr. Eva G. Campbell, Guilford College.

For Secretary-Treasurer: Dr. Bert Cunningham, Duke University.

For Member of Executive Committee: Prof. O. J. Thies, Jr., Davidson College.

For Member of Research Grants Committee: Prof. J. P. Givler, Woman's College U. N. C.

For Representative on Council A. A. A. S. and the Academy Conference: Bert Cunningham, Duke University.

James B. Bullitt
P. M. Ginnings
John S. Holmes, *Chairman*

After the reading of this report the President called for nominations from the floor. It was moved, seconded, and carried that nominations be closed and that J. B. Bullitt be instructed to cast an unanimous ballot for the nominees.

Dr. Bullitt reported that such a ballot was cast.

Report of the Resolutions Committee

The North Carolina Academy of Science wishes to express its appreciation for the pleasant entertainment received at the Woman's College of the University of North Carolina. The local committee has worked most efficiently to make us

enjoy our visit, through the excellent facilities provided for the meeting, the splendid exhibits, the complimentary dinner, and the general good fellowship. We wish also to express our appreciation to Dean W. C. Jackson and the administration of the college, also to the Greensboro Chamber of Commerce and Mr. J. S. Patterson for the interest shown in the visit of the Academy and to Miss Craig who handled our press notices so efficiently.

W. C. GEORGE
C. H. HIGGINS
E. G. CAMPBELL, *Chairman*

The resolution was adopted.

The Academy then adjourned for the complimentary dinner provided by the Woman's College.

President Coker announced the winners of the High School exhibits award. They were Ernest Hardwicke and Arthur Budlong who exhibited jointly a home-made stroboscope. Other exhibits which should be especially mentioned were (1) an unusual group of photographs presented by Donald Hartzog of the Winston-Salem High School; (2) Indian arrow heads collected by Edgar Hayes of the High Point High School; and (3) Miniature replicas of fire arms made by Joe Fidel of the High Point High School.

The Academy met in its customary night session to hear the Presidential Address. A. D. Shaftesbury, Chairman of the local general committee, presided at this meeting in the absence of C. N. Warfield, Vice-President of the Academy, who was absent on account of war duties. The Chairman introduced Dean W. C. Jackson of the Woman's College who extended a welcome to the Academy. R. E. Coker, President of the Academy, then delivered his Presidential Address. At the close of the meeting refreshments were served and a social hour enjoyed. Perhaps the "black-out" in the middle of this social affair added to its zest. The feeling prevalent as the party broke up was that we had all had a pleasant and profitable day.

The various Sections met on Saturday for sessions of variable lengths. All had good programs and unexpectedly large attendance. The new section on Wildlife was especially gratifying.

At the request of several members of the Academy the Secretary is giving here a list of the recent meeting places of the Academy:

Greensboro, 1935; Duke, 1936; Catawba, 1937; State, 1938; Wake Forest, 1939; Davidson, 1940; Carolina, 1941; Greensboro, 1942.

The personnel of the standing committees follows:

Executive: H. F. Prytherch, Eva G. Campbell, Bert Cunningham, A. S. Pearse, D. B. Anderson, O. J. Thies.

Research Grants: J. N. Couch (1944), Chairman, O. C. Bradbury (43), H. D. Crockford (43), C. F. Korstian (44), J. P. Givler (45).

The following sectional officers were elected by the respective groups:

Botany: Chairman, Earl H. Hall; Secretary, E. C. Cocke (45). Attendance, about 45.

Biochemistry and Physiology: Chairman, C. Artom; Secretary, J. C. Andrews (45). Attendance, about 15.

Geology: Chairman, H. T. Davis; Secretary, Willard Berry (45). Attendance, about 15.

Mathematics: Chairman, none elected; Secretary, J. W. Lasley, Jr. (43). No meeting.

Physics: Chairman, A. L. Hook; Secretary, F. W. Lancaster (45). Attendance, about 25.

Psychology: Chairman, K. L. Barkley; Secretary, Karl Zener (45). Attendance, about 40.

Wildlife: Chairman, Willis King; Secretary, R. O. Stevens (45). Attendance, 35–40.

Zoology: Chairman, O. C. Bradbury, Secretary, Eva G. Campbell (45). Attendance, about 30.

High School Science: Chairman, J. W. Wood; Secretary, Nellie D. Blackburn. Attendance, 15.

President H. F. Prytherch announces the following committees to serve during 1942–43.

Auditing Committee:

Speas, W. E., *Chairman*, Wake Forest College, Wake Forest, N. C.
Bradbury, O. C., Wake Forest College, Wake Forest, N. C.
Mackie, G. C., Wake Forest College, Wake Forest, N. C.

Conservation Committee:

Holmes, J. S., *Chairman*, State Forester, 302 Forest Road, Raleigh, N. C.
Baity, H. G., Chapel Hill, N. C.
Korstian, C. F., Forestry, Duke University, Durham, N. C.
Gordon, Seth, Jr., N. C. Department of Conservation & Development, Raleigh, N. C.
Brimley, H. H., State Museum, Raleigh, N. C.

High School Science Committee:

Buell, M. F., *Chairman*, State College, Raleigh, N. C.
Braun, M. L., Catawba College, Salisbury, N. C.
Dodson, C. F., Western Carolina Teachers College, Cullowhee, N. C.
Highsmith, J. H., Department of Education, Raleigh, N. C.
DeLoach, W. S., East Carolina Teachers College, Greenville, N. C.
Conner, Elizabeth, High Point, N. C.

Legislative Committee:

Berry, Willard, *Chairman*, Duke University, Durham, N. C.
Wells, B. W., State College, Raleigh, N. C.
Burgess, B. C., United Feldspar and Minerals Corp., Spruce Pine, N. C.
Rosenau, M. J., University of North Carolina, Chapel Hill, N. C.

Necrology Committee:

Beers, C. D., *Chairman*, University of North Carolina, Chapel Hill, N. C.
Brandt, B. B., East Carolina Teachers College, Greenville, N. C.
Wyatt, W. J., Jr., Wake Forest College, Wake Forest, N. C.

Bookout, C. J., Duke University, Durham, N. C.
West, Gladys F., State College, Raleigh, N. C.
Sprague, A. D., Elon College, N. C.
Barrow, E. E., W. C. U. N. C., Greensboro, N. C.
Arbuckle, H. B., Davidson College, Davidson, N. C.
Ullman, R. R., Lenoir Rhyne College, Hickory, N. C.
Cleaver, Mrs. W. J., Catawba College, Salisbury, N. C.

Nominating Committee:
Coker, R. E., *Chairman*, University of North Carolina, Chapel Hill. N. C.
Wolf, F. A., Duke University, Durham, N. C.
Stuckey, J. L., State College, Raleigh, N. C.

Poteat Award Committee:
Shaftesbury, A. D., *Chairman*, W. C. U. N. C., Greensboro, N. C.
Gray, Irving E., Duke University, Durham, N. C.
Dashiell, J. F., University of North Carolina, Chapel Hill, N. C.
Braun, M. L., Catawba College, Salisbury, N. C.
Bradbury, O. C., Wake Forest College, Wake Forest, N. C.
Hood, Frazer, Davidson College, Davidson, N. C.
Satterfield, G. H., State College, Raleigh, N. C.
MacCarthy, G. R., University of North Carolina, Chapel Hill, N. C.
Kramer, P. J., Duke University, Durham, N. C.

Committee on Resolutions:
George, W. C., *Chairman*, University of North Carolina, Chapel Hill, N. C.
Metcalf, Z. P., State College, Raleigh, N. C.
Gravette, H. L., Elon College, N. C.

Committee on Primeval Forest:
Holmes, J. S., *Chairman*, 302 Forest Road, Raleigh, N. C.
Wells, B. W., State College, Raleigh, N. C.
Bradbury, O. C., Wake Forest College, Wake Forest, N. C.
Oosting, H. J., Duke University, Durham, N. C.
Thiel, A. F., W. C. U. N. C., Greensboro, N. C.
Weil, Lionel, 611 Park Avenue, Goldsboro, N. C.
Spencer, Colin J., N. C. Forestry Assoc.

Junior Academy Committee:
Dodson, C. F., *Chairman*, Western Carolina Teachers College, Cullowhee, N. C.
Heck, C. M., State College, Raleigh, N. C.
Hall, E. H., W. C. U. N. C., Greensboro, N. C.
Wilson, Dorothy, Durham High School, Durham, N. C.
Howe, M. D., Queens College, Charlotte, N. C.

Life Member Qualification Committee:
Pearse, A. S., *Chairman*, Duke University, Durham, N. C.
Ferrill, H. W., University of North Carolina, Chapel Hill, N. C.
Satterfield, G. H., State College, Raleigh, N. C.
Mackie, G. C., Wake Forest College, Wake Forest, N. C.
Arundel, Miss Edna, W. C. U. N. C., Greensboro, N. C.

Constitutional and Secretarial Committee:

Totten, H. R., *Chairman*, University of North Carolina, Chapel Hill, N. C.
Blomquist, H. L., Duke University, Durham, N. C.
Cunningham, Bert, Duke University, Durham, N. C.

The following papers were presented during the meeting. Those marked with an x are abstracted in the Proceedings; those marked with an * are published in full.

GENERAL SESSIONS

Address of Welcome. W. C. JACKSON, Dean of Administration of the Woman's College of the University of North Carolina

Presidential Address. What are the Fittest? R. E. COKER, President of the Academy

Strawberry growth as affected by mineral nutrition. R. A. LINEBERRY AND LELAND BURKHART, Dept. of Agric.

x*Important North Carolina raw materials and manufacturing facilities available for war use.* E. E. RANDOLPH, State.

x*An abnormally large chicken liver.* E. C. COCKE, Wake Forest.

x*The pulling strength of nails of different size and depth.* J. B. DERIEUX, State.

x*Growth and nutrition of the peanut plant.* LELAND BURKHART, State.

Tropical opossums (colored film by Wistar Institute). A. D. SHAFTESBURY, Woman's College.

Vitamin C in strawberries and other small fruit grown in North Carolina. LELAND BURKHART, R. A. LINEBERRY, Dept of Agric.

Factors inhibiting the invasion of spruce-fir forest by maple-basswood reproduction and ground cover. M. F. BUELL AND W. E. GORDON, State and Minnesota.

x*The fauna of sand beaches at Beaufort.* A. S. PEARSE, Duke.

x*Ecological studies of bacteria of ocean beaches at Beaufort, N. C.* H. J. HUMM, Duke.

x*Construction and operation of a solar heating unit.* L. B. RHODES, N. C. Dept. of Agric.

x*More detailed studies of the meteorological contrasts of day and night.* C. M. HECK, State.

Ecological studies of virgin forests. H. J. OOSTING, Duke.

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*x*A study of the gross physiological reactions of lower organisms to the antisulfonamide, para-amino-benzoic acid.* E. M. LAVOR, F. F. FERGUSON, AND V. VAN ALDERMAN, Norfolk General Hospital, W. and M., and V. P. I.

x*Variations in the nature of soil colloids of North Carolina and their relation to plant growth.* A. MEHLICH AND F. M. MILAM, State.

Nicotinic acid in milk. E. A. BAILEY, JR. (in collaboration with G. H. SATTERFIELD, W. J. DANN, C. D. GRINNELLS), State and Duke.

x*The enzymatic decomposition of quinine in vitro and in vivo.* JAMES C. ANDREWS AND C. E. ANDERSON, Carolina.

A porometer study of cotton stomates. B. F. VOLKERDING AND D. B. ANDERSON, State.

Action of brominated fatty acids on liver fat.. C. ARTOM AND M. SWANSON, Wake Forest.

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Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture, Raleigh.

3. *Heteropoly-acids of Columbium and Tantalum.* F. H. EDMISTER, University of North Carolina and C. P. TEBEAU, University of Georgia.
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7. *The structures of cellulose; mercerization.* SHERMAN E. SMITH, University of North Carolina.
8. *Reactions of atomic hydrogen.* F. H. EDMISTER, University of North Carolina and GEORGE L. CHURCH, Philadelphia Salt Company.
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Important North Carolina Raw Materials and Manufacturing Facilities Available for War Use. E. E. RANDOLPH.

At this critical time when the very existence of our government and all that it means to our freedom of thinking and action are at stake, it is necessary that all of our physical and mental ability, all of our materials, and all of our energies are put to the utmost use to defend and preserve our freedom and our principles of life.

We should explore to the limit our natural resources, our necessary materials, our present manufacturing advantages and facilities, and our opportunities for making other necessary materials and the making of those resources available for the present crisis. In this effort we should of course as far as possible create industries which may later contribute to permanent advantage of our people. On the other hand if we have vital materials which the government needs we should find a way to make them available even if it should be inadvisable to continue the enterprise after the war.

We should investigate the advisability of working our tin ore for immediate production. Our copper operations might be increased. New mica areas of the state could be producing. Our iron deposits have been profitably worked and are still a potential source for iron. Our lime deposits could be used to produce cement. Although our five pulp mills are running to capacity, our forests under proper control could furnish timber for another. Our chemical industries are now one of our principal financial manufacturing agencies, producing such necessary materials as fertilizers, pulp, paper, aluminum, bromine, leather, rayon, heavy and fine chemicals, edible oils and fats, and metals. We have all the opportunities for greatly increasing our part in manufacturing necessary chemicals for war purposes. In addition to a proposed ordnance works we could be producing explosives. We have extensive coal deposits which can be worked and farm products can be increased.

Because of the freedom of private enterprise our sources of power have been well developed in this state so that we have available all power required for our industries and war needs and also extra power to take care of industries in other states through our super-power systems.

Some of our enterprising small plants and machine shops are sub-letting contracts of large companies to produce parts of machines necessary for war purposes. Careful study should be made that all of our facilities may be used to the best advantage at this time to hasten the winning of the war and the assuring of a reasonably early victory.

An Abnormally Large Chicken Liver. E. C. COCKE.

An abnormally large chicken liver was described. The liver was removed from a young Plymouth Rock pullet. The chicken weighed 3¾ pounds alive, the liver weighed 13½ ounces, which was about five times the size of a normal chicken liver.

Pulling Strength of Nails of Different Size and Depth. J. B. DERIEUX.

Previous to the 19th Century, nails were made by hand in the households of England as an art and trade. The first nail-making machine was invented by an American in 1786.

The sizes of nails are designated in penny. The origin of the word "penny" as applied to the size of nails is not very well known. Some authorities believe it originated from the cost in English pence of a hundred of the nails, others believe that it originated from the weight in pennyweight of a hundred of the nails, and still others believe that the word "penny" is a change of the word "pun", which was used for the word "pound".

The lengths of nails increase ½ of an inch for each increase in size, beginning with the 2-penny at one inch, up to the 10-penny, from which point it increases one inch for each of the next four sizes, 10, 20, 40, and 60, the 60 being 6 inches long. The diameter of the 2-penny is .072 inches and for the 60-penny is .263 inches. The number per pound is about 1,000 for the 2-penny and 10 for the 60-penny, or the weight per 1,000 nails is almost one pound for the 2-penny and 100 pounds for the 60-penny.

The study was made on common wire nails from size 2-penny to 60-penny inclusive. They were driven into medium green oak by increasing depths of ½ of an inch, and pulled after each increase, until the entire length of the nail was imbedded. The pulling was done by a lever system, the force to pull the nails being computed from the force at the end of the lever and the lever arms. The maximum pulling strength of the 2-penny, which is one inch long, was about 200 pounds and that for the 60-penny was almost 4,000 pounds. The strength for each sized nail varied directly as the depth. The strength for the same depth, one inch, varied approximately as the diameter of the nails, and the area for a given depth varying as the diameter of the nails, the pulling strength thus varied as the area of contact.

Growth and Nutrition of the Peanut Plant. LELAND BURKHART.

Calcium is established as an indispensable nutrient throughout the growth of the peanut plant. Employing the sand culture technique, mineral nutrients other than calcium in the peanut kernel are relatively mobile and are readily reutilized. Foliar deficiency symptoms of calcium, potassium, magnesium, phosphate, nitrogen and boron, respectively, and associated foliar nutrient levels are established for the Virginia Bunch peanut plant. The phosphate concentration must be kept low in sand cultures to prevent injury. Foliar diagnosis of nutrient conditions in young field-grown plants with special reference to the calcium-potassium-magnesium relationship is of practical value. The peanut fruit develops in the soil and, therefore the chemical, physical, and biological conditions in soils have a special significance in directly affecting quality of peanut fruits with particular reference to the formation of inferior fruits or so-called "pops" by the large type peanut varieties. A new approach for determining the specific effects of calcium and potassium on fruit quality is described. The fruiting medium was isolated from the rooting medium. Calcium was found to be very beneficial and necessary in the fruiting medium for the production of good fruit, irrespective of the nature of the rooting medium. The unfavorable effect of potassium in the fruiting and rooting media was largely overcome by the accompaniment of calcium in the fruiting medium. The absorption and translocation of minerals from the fruiting medium by growing peanut fruits was exemplified by lithium intake and distribution in the plant. The interaction of the rooting medium on the quality of fruit formed in the fruiting medium is clearly demonstrated.

The Fauna of the Sand Beaches at Beaufort. A. S. PEARSE and G. W. WHARTON.

Marine sand beaches are not barren, shifting wastes, but teem with animal life. At Beaufort, besides various macroscopic molluscs, crustaceans, and other peculiarly adapted psammobionts, there are in each liter of inter-tidal sands about 400 nematodes, 300 copepods, 60 foraminiferans, 35 ostracods, and many other minute animals. When tides ebb, shore birds forage over beaches; when they flow, schools of little fishes come to get their share of the great food resources that are present in sandy shores.

(The complete paper is included in Ecological Monographs Vol. **12**, No. 2.)

A Study of the Role and Importance of Bacteria in Ocean Beaches at Beaufort, North Carolina. H. J. HUMM.

Ocean beaches contain many more bacteria per unit volume of sand than might be expected and these organisms play a much more important ecological role than is generally realized. Organic matter is continually washed up, decomposed by bacteria, and the minerals returned to the sea. A remarkable variety of physiological types of bacteria make up the beach population. Among these are agar, chitin, and cellulose digesters; nitrite and nitrate reducers, denitrifiers, and probably nitrifiers and nitrogen-fixers.

On the outer Ft. Macon beach at Beaufort, an average of all samples taken was 200,000 per gram of sand. At the low tide mark, the average was 34,000; at mid-tide, 110,000; at high tide or drift line, 486,000. Counts of agar-digesters ran from 200 to 15,000 per gram. These figures probably represent only from 70 to 90% of all saprophytic, aerobic bacteria which would form macroscopic colonies on the medium employed after 5 to 7 days' incubation. The highest count for one gram of sand, 2,250,000, was from a high tide sample.

(The complete paper is included in Ecological Monographs Vol. 12, No. 2.)

Construction and Use of a Solar Heating Unit. L. B. RHODES.

An experimental Solar Heating Unit is constructed and installed in practical use for observing characteristics in operation under Raleigh weather conditions. Twenty-six (26) $\frac{3}{8}$" copper tubes are soldered $1\frac{1}{2}$" apart in equi-spaced holes in two (2) 1" brass pipes so that each 1" pipe forms parallel headers. Heavy sheet copper strips are soldered the length between all the $\frac{3}{8}$" tubes. The whole unit employs two such sections, all having a total area for direct sun's rays of 23.5 square feet.

With the headers of each section joined opposite and the sections in the same plane, the assembly is tilted 18 degrees from horizontal and toward the sun. The water supply enters the bottom header from a storage tank passing through all tubes to the top header and thence to the top of an inside 40 gal. tank. Natural convection produces continuous circulation of the water. All copper surfaces exposed to the sun's rays are black nickel plated and oiled which, it was discovered, increased efficiency of heat transfer.

The unit, which is located on the roof top, is enclosed in a cabinet heavily insulated with reflective surfaces. All pipes to the tank are heavily insulated with thick layers of cotton. The cabinet is covered by ordinary window glass. Underneath, electrical heating units are available for protection of the units from freezing, but are used only at temperatures around 6 degrees F.

The heated water passes from the tank into an electric water heater for distribution. The first tank, though uninsulated, holds its temperature with moderate loss overnight. The efficiency of the unit is about the same in winter as summer, usually heating 3 to 4 tanks, a rise of 60 to 65 degrees F. Highest temperature observed is 138 degrees F. of the tank on bright days. The equivalent in work of the energy collected, neglecting the daily heat loss from the tank per day of six (6) hours bright weather, is 12.7 to 14 K.W.H. or 381 K.W.H. upward per month which compares with 12 K.W.H., the average radiant energy available on an equal surface for six (6) hours at the average rate of 1.3 gram calories per sq. cm. per minute.

January, 1942, showed 79.1% bright days
February, " " 70 % " "
March, " " 75 % " "

October, 1941, to January, 1942, showed six (6) days too overcast for any useful radiation.

A More Detailed Study of the Meteorological Contrasts between Day and Night. CHAS. M. HECK.

To avoid the dampening effect of the large heat mass found in the standard U. S. Weather Bureau wooden shelter, an exact reproduction in size and shape was made in thin aluminum with double walls. This reduced the heat mass to one fifth and temperature changes in the atmosphere were recorded more exactly. However the maximum in the day was always higher in the aluminum shelter averaging about five degrees. More surprising was the unexplained phenomena of a higher minimum also. This averaged one degree. Since climatic records are made by averaging the maximum and minimum each day, Raleigh's climate would be recorded as three degrees warmer if the Weather Bureau used aluminum shelters of this type. Further the diurnal average change would be recorded as 23°F instead of 19°F. The arbitrariness of temperature recordings in wooden shelters is thus made evident and the need for more scientific standardization of atmospheric temperature records is indicated.

Using the same system of climatic recording, electrical records over a number of weeks in spring gave the diurnal maximal difference at the earth's surface on top of two dead leaves to be about three times that in the free air three feet above the leaves. This and other phenomena such as relative humidity, motion of the air, dew and frost were cited to indicate the great differences presented in worm's-eye-view meteorology from the meteorology that man records from his own standpoint.

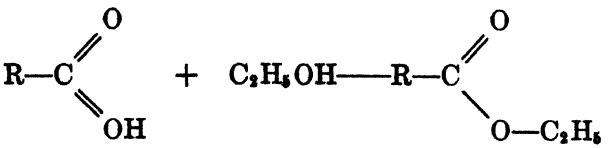
A Study of the Gross Physiological Reactions of Lower Organisms to the Antisulfonamide, para-amino-benzoic acid. E. M. LAVOR, F. F. FERGUSON and V. VAN ALDERMAN.

This is a generalized survey of the reactions of pathogenes, non-pathogenes, *Amoeba*, various ciliates and rotifers to aqueous solutions of the common sulfonamides and para-amino-benzoic acid and to combinations of the same. The work is outlined to: (1) ascertain the toxic and lethal effects of PAB and various sulfonamides upon laboratory cultured *Paramecium*; (2) find out if a non-toxic dilution of PAB nullifies the slightly toxic dilutions of a sulfonamide when used on *Paramecium*; and (3) inquire into the value of PAB as a growth promoting factor. Present findings: (1) using dilutions ranging from 0.09% to 0.01% of PAB *alone* we find that it is definitely toxic in dilutions *above* 0.02% and entirely lethal *above* 0.05% as used upon *Paramecium*; (2) using dilutions ranging from 0.8% to 0.08% of sulfanilamide *alone* we find that it is either toxic or lethal in its effect upon *Paramecium* in dilutions *above* 0.32%; (3) the slight degree to which PAB may act as an antisulfonamide in a combination with sulfanilamide is not in this case convincing; (4) at the onset in toxic effects of sulfanilamide upon *Paramecium* the weaker specimens succumb almost instantly, the others move slowly and swell appreciably, in higher concentrations producing pellicle papillae. A secondary effect is specimen immobility upon the substratum, later normal activity and appearance resumes for most; thus toxic effects do seem to be gradually thrown off.

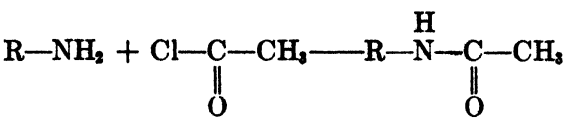
A Study of the Sulfonamide Inactivating Groups of Para-amino-benzoic Acid. V. VAN ALDERMAN, EDWARD M. LAVOR, and FREDERICK F. FERGUSON. (Read by title.)

We are attacking the problem of ascertaining the antisulfonamide group or groups in PAB by the following trial approaches:

1. Block off the carboxyl (acid) group by esterification.



2. Block off the amino (basic) group by acetylation:



The resulting compounds will be tested against various pathogenes, non-pathogenes and lower invertebrates for physiological reactions.

Variations in the Nature of Soil Colloids of North Carolina and their Relationship to Plant Growth. A. MEHLICH and F. M. MILAM.

The relationship of percentage base saturation and pH when measured over a wide range of reaction was found to be a specific expression of the nature of the base exchange mineral present in soil. These determinations are made rapidly since they are carried out with the natural soil. A large number of data pertaining to variations in the nature of colloids of North Carolina soils are being made available. This information is useful as an aid in the classification of soil particularly where the nature of the base exchange colloid plays a leading role in the mapping of soil types. It is important in problems of liming and fertility.

Results with peanuts for example have shown increasing hay yields by increasing the base saturation from 25 to 75 per cent when these plants were grown in sand culture containing an organic colloid. When peanuts were grown in a sand culture containing a kaolinitic colloid the yield of hay was about the same for 25, 50, and 75 per cent base saturation respectively. No peanuts were produced on a soil with less than 13 per cent base saturation and best yields were generally obtained on soils having 50 per cent or greater, base saturation.

The importance of percentage base saturation and nature of soil colloids on the absorption of mineral nutrients by tobacco has been shown. Calcium and potassium are usually absorbed more readily from the kaolinitic type of colloid than from the montmorillonitic or organic colloid.

The Enzymatic Decomposition of Quinine in Vitro and in Vivo. JAMES C. ANDREWS and CARL E. ANDERSON.

Lipkin, in 1919, suggested that quinine is enzymatically destroyed by the minced tissues of certain organs of the guinea-pig, rabbit, sheep, and ox. Using rats, we have confirmed this statement and have studied the distribution of the enzyme. We have also made some preliminary studies of the nature of this enzymatic reaction. Minced liver, kidney, brain and lung destroy quinine upon incubation with the latter for 24 hours at 40°C. The criterion of destruction of the quinine consisted in its apparent disappearance as estimated by the method of Kyker, Webb, and Andrews. The highest degree of activity was shown by minced liver. Attempts were therefore made to prepare a more concentrated enzyme preparation from fresh rat livers. By extraction of the minced tissue with 50% alcohol and precipitation at 70% of the same we have obtained a crude, active preparation of the enzyme.

The activity of the liver in the destruction of quinine was demonstrated *in vivo* as follows: The excretion of quinine by young adult rats after standard dosage was measured and found to vary between from 5 to 14% of that administered. After removal of from 30 to 50% of the liver the quinine excretion increased by 100 to 200% of the normal. After these rats were allowed to "regenerate" their livers the quinine recoveries fell to the normal range. On completion of the experiment and as a control against possible influence of operative shock, some of the rats were splenectomized and in others an abdominal incision was made. Quinine recoveries were again in the normal range.

This enzymatic conversion of the quinine lends support to the hypothesis that the effective anti-malarial agent is probably a metabolic product of quinine.

Further Notes on the Reactions of Certain Lower Organisms to the Common Sulfonamides. EDWARD M. LAVOR and FREDERICK F. FERGUSON.

The following information is furnished by this recent study:

1. Sulfaguanidine is not as uniform in its toxic action upon free-living microfauna as are sulfanilamide, sulfathiazole, and sulfapyridine.

2. While displaying a uniform lack of toxicity in *higher* concentrations, sulfaguanidine may at times show a definite toxicity in *lower* concentrations.

3. Toxic effects of sulfaguanidine do not appear uniformly as far as time is concerned.

4. *Mesostoma* sp. when exposed to sulfathiazole exhibits the expected toxic reactions plus an unusual increase in parenchymal pigmentation. This form is very susceptible to toxic effects of sulfanilamide.

Panicum Bennettense, a New Species from North Carolina. WALTER V. BROWN.

Three plants of this new species were collected at the Bennett Memorial, Durham, N. C. Although much like *P. angustifolium* Ell. it differs in the shorter spikelets, 2 mm., in the shape of the spikelet, elliptic, in the shorter first glume, ½ the length of the spikelet, and in the longer ligule. It is obviously

closely related to *P. angustifolium* and belongs in the group Angustifolia. The type specimen has been deposited in the Duke University Herbarium.

Investigations of Control Measures for Granville Wilt of Tobacco. T. E. SMITH.

Experiments were started in 1935 to develop more effective control measures for Granville wilt (*Bacterium solanacearum* E. F. Smith) of tobacco. It has been shown that losses from wilt are considerably reduced by the growth of tobacco after three years of either corn, soybeans, or red top grass. However, crop rotation appears inadequate for ultimate control of this disease. Soil treatment with chloropicrin has given effective control but this is too expensive for use on large areas in the field. The combination of a corn rotation with urea treatment of the soil (1000 pounds per acre of Uramon) gave promising results. A high degree of genetic resistance was found in a collection of tobacco from Colombia, South America, and progress has been made by hybridization and selection toward the development of resistant varieties of flue-cured tobacco. When all phases of the work are considered, it seems that highly effective control measures are now in prospect.

A Discussion of Olpidiopsis and Pseudolpidium. ALMA J. WHIFFEN.

Though the genera Olpidiopsis and Pseudolpidium have been combined because a monospore culture of a species of Olpidiopsis produced both resting bodies with companion cells and resting bodies without companion cells, the question has remained as to whether or not there actually exist species of Olpidiopsis in which the resting bodies always lack companion cells as has been described. *Pseudolpidium gracile* on *Pythium rostratum* was studied and Butler's observation that this species lacks companion cells on the resting bodies is confirmed. *Aphanomyces cladogamous* was found to be parasitized by a species of Olpidiopsis, the resting bodies of which lack companion cells and which is recognized as *O. Aphanomycis* Cornu. Two new species of Olpidiopsis, both having companion cells on the resting bodies, are described as *O. curvispinosa* and *O. brevispinosa.* Both of these new species are parasites of Pythium. The conclusion is that in the genus Olpidiopsis there are species in which the resting body is constantly present on the resting body and species in which it is constantly lacking.

Osmotic Pressure and Diffusion Pressure Deficit Relations in Cotton Bolls. D. B. ANDERSON and THOMAS KERR.

Studies of the osmotic pressure and water-absorbing capacity of growing cotton seeds indicate that the force with which water enters the seeds may greatly exceed the osmotic pressure of the seeds. This fact is contrary to the widely held theory that water movements are determined as to direction by the relative diffusion pressures of the cells and suggests that energy is expended by the cells in the process of water absorption. The water-absorbing power of the carpel wall of cotton bolls of all ages invariably exceeded the osmotic pressure of the cells. In growing seeds the O. P. exceeded the water-absorbing power

when the seeds were young but fell below the water-absorbing power when the seeds were older than twenty-four days. The relative values of osmotic pressure and water absorbing capacity are indicated graphically.

Some Blue-green Algae Which Have Not Been Reported for North Carolina. ELTON C. COCKE and F. E. LEATHERWOOD.

Thirty-four species of blue-green algae new for North Carolina are reported and one species which is believed to be new to science. These algae were collected in the vicinity of Wake Forest and in Haywood County. The new species is tentatively placed in the genus *Dactylococcopsis* and given the new specific name *Poteatii* in honor of the late Dr. W. L. Poteat.

The Effect of Light Intensity on Photosynthesis in Pine and Oak Seedlings. PAUL J. KRAMER and JOHN P. DECKER.

The rate of photosynthesis of potted seedlings of loblolly pine (*Pinus taeda* L.) and eastern red oak (*Quercus borealis maxima* (Marsh) Ashe) were measured at seven light intensities ranging from 300 to 9300 foot candles. These intensities were obtained with Mazda reflector-type bulbs and various screens. The air temperature was maintained at 25° C. The apparent photosynthesis was measured in terms of absorption of carbon dioxide by the entire shoot. Maximum photosynthesis of oak seedlings occurred at about 3300 foot candles and photosynthesis decreased slightly at higher intensities. Photosynthesis of pine seedlings increased with light intensity up to the highest light intensity used, 9300 foot candles.

Some Additions to the Dicotyledonous Flora of South Carolina. BUDD E. SMITH.

Since 1932 I have been studying the flora of Darlington County, South Carolina. The county lies within the Coastal Plain except for the western edge which is in the lower Piedmont. The Great Pee Dee River forms the eastern boundary of the county, and in the southeast portion of the county two islands are surrounded by backwaters from the river. The river has its origin in the mountains of western North Carolina as the Yadkin River. The largest of the islands is called Witherspoon Island and a study of the plants found on the island has added many species to the flora of the state. One thousand and seventy-six (1076) species and varieties of dicotyledons have been identified for the county, and after checking all available literature and herbaria possible we find that the following 37 species are new to the flora of South Carolina:

Froelichia floridana
Gomphrena decumbens
Stellaria fontinalis
Portulaca pilosa
Rubus Baileyanus
Amelanchier stolonifera
Amelanchier arborea
Rhynchosia mollissima
Tephrosia Rugelii
Vaccinium liparius
Vaccinium holophyllus
Cochranea anchusaefolia
Lamium purpureum
Scutellaria Altamaha
Ilysanthes grandiflora
Penistemon pallidus
Ruellia humilis
Ruellia hybrida

Tragia linearifolia
Chamaesyce Blodgettii
Chamaesyce hirsuta
Chamaesyce humistrata
Chamaesyce conferta
Euphorbia zinniiflora
Hypericum glomeratum
Isnardia spathulata
Lechea prismatica
Rhexia Nashii
Richardia brasiliensis
Diodia rigida
Sherardia arvensis
Melothria crassifolia
Specularia leptocarpa
Laciniaria Garberi
Tagetes minuta
Chrysopsis oligantha
Vernonia recurva

An Unusual Brown Iron Ore Deposit. J. W. HUDDLE.*

There is an unusual brown iron ore deposit in the southwestern portion of Cleburne County and the northwestern portion of Clay County southwest of Heflin, Alabama. These counties are in the Piedmont Plateau Province and are underlain by metamorphic rocks most of which were originally sedimentary. The ore occurs in the upper portion of the Talladega Series in a strongly sheared chloritic graphite schist. The deposits of proven or probable commercial value lie in a narrow zone along the south flank of a ridge held up by a quartzite member of the Talladega Series. The ore is not continuous in commercial quantities, but near the contact of the deeply weathered chloritic graphite schists and quartzites some ore was found for a distance of several miles. Much of the ore is low grade, but at several places the ore is high grade and occurs in large irregular bodies elongated in the direction of strike. It ranges in texture from solid to porous and massive to concretionary. Exposures of the ore body at the Chulafinnee Ore Company pit show the ore to replace the chloritic graphite schist. The Chulafinnee Ore Company mined over 130,000 short tons of ore from a body extending unbroken for about 1,800 feet with a thickness averaging about 30 feet. This ore averaged about 50% iron, .85% phosphorus, 4.0% aluminum, .60% manganese, 13.0% insolubles, 8.0% as received water. The largest deposit in the zone has not yet been extensively developed, largely because the phosphorus content exceeds 1%. This ore body is known to extend a half mile in unbroken deposit with a considerable thickness.

Nearly all the brown iron ore deposits of the Southern Appalachians are in residual clays derived from the weathering of iron bearing rocks. The ore occurs in pockets separated by extensive barren zones. The ore deposits here discussed are unusual in the great extent of unbroken deposits and in the fact that they are replacement rather than residual deposits. Some of the iron may have been derived from the hematite in the quartzites immediately adjacent to the chloritic graphite schists, and it is possible that the weathering of pyrite in other members of the Talladega Series may have supplied some iron. There is no indication that the deposit is a gossan. The concentration of the ore bodies seems to be best explained by cross-faulting, since the whole zone is strongly sheared. The deep weathering of the schists and quartzites is probably related to the formation of the ore, and it is possible that they were formed at the same time as the bauxite and primary kaolins of southeastern United States.

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Reviewing North Carolina Coastal Plain Geology. HARRY T. DAVIS and HORACE G. RICHARDS.

For some two years now the North Carolina State Museum and the Academy of Natural Sciences of Philadelphia, have carried on cooperative studies, with field work, of eastern North Carolina.

Previous studies and publications were excellent, but necessarily incomplete in details. We would gather all studies for a more comprehensive coverage, possibly a new publication.

Our field work has added numerous fossil localities and new fossils from the Cretaceous to the Pleistocene. The most significant of the latter have come from the Castle Hayne (Eocene) and the Trent (Miocene). We are compiling records of the associated economic resources such as marl, phosphates, building stone, ilmenite, clays, water supply, etc., that are so important at this time.

We expect this survey to clarify many of the perplexing problems that students of this subject have pondered.

Economic Geology of the North Carolina Peridotites. T. G. MURDOCK.

North Carolina pegmatites have made an important contribution to the mining industry in the state. It is expected that the peridotites will make, in the future, a similar and even greater one. In North Carolina and Georgia more than 275 peridotite formations occur; many of them are dunites. A recent survey indicates that there are 20 olivine deposits containing an aggregate of at least 230,000,000 tons of material averaging 48.07% magnesia. Olivine deposits are of the dunite and saxonite types. Structurally the intrusives occur as lenses and ring dikes. Olivine production to date has been on a small scale, principally for refractory use; a plant at Webster has produced magnesium sulphate. Research is being conducted toward utilization of olivine as a source of magnesium, but prior work, and other factors, have led to more consideration being given to other sources, particularly sea water. Vermiculite has been produced from several peridotite areas; a small industry has been developed in Macon and Buncombe counties. This unique group of minerals is finding an important place as a light-weight insulating material. Chromite occurs at several localities, usually in small quantities; but it does have an economic importance and placer mining was carried out at Democrat in 1941. Nickel-bearing minerals occur at several localities. Anthophyllite occurs in many of the dunites and production has been reported from Avery County. Further geological studies will obtain much useful information regarding these most interesting formations and eventually lead to a wider development.

The Geomagnetic Disturbance of August 4, 1941, as Observed in North Carolina and in Oregon. G. R. MACCARTHY.

During the mild "magnetic storm" of August 4, 1941, identical magnetometers were being operated at Johnson Creek, Oregon, and at Webster, North Carolina. Although these localities are approximately 37° 7′ apart in longitude, and in regions which are geologically quite different, there is very little difference in the records made at the two stations. In each case the vertical

component of the earth's magnetic field increased at a nearly constant rate during the period of observation, the rate being 17 gammas per hour at Webster, and 16 gammas per hour at Johnson Creek.

Geology of Three Wells in the Coastal Plain of North Carolina. WILLARD BERRY.

The need for potable water in the several defense areas in eastern North Carolina caused an increase in drilling in that area. Mr. M. J. Mundorff of the United States Geological Survey in cooperation with the State Geological Survey collected samples from these wells. Three of the wells in a more or less east west line have been examined in detail.

The Lane Housing Project well was drilled west of Jacksonville, Onslow County, and is the farthest west. The Rural Electrification Administration well is on the east side of the Northeast Creek at the R. E. A. power station on N. C. Route 24, Onslow County. Cherry Point #2 well is at the Marine Base at Cherry Point on the Neuse River in Craven County and is the farthest east of the three.

The section encountered in the Lane Housing Project well is Pleistocene 0–34 feet, Pliocene 34–47 feet, and Miocene from there to bottom, that from 109 to 132 is probably Trent Marl. The R. E. A. well section would seem to be Pleistocene 0 to 58 feet, Pliocene 58 to 88 feet, Miocene 88 to 257 feet (possibly Duplin), and 257 to 327 (probably Trent Marl). Below 327 probably Eocene and still in Eocene at 566 feet. Minor breaks between 367 and 388, 391 and 395, 403 and 445, and 445 and 566. This well drilled to 588 and ended with salt water but last sample is labeled 566. Cherry Point well #2, Pleistocene 0–35 feet, Pleiocene 35–70 feet and Miocene 70 to 140 feet which is probably Trent Marl. These wells lack definite evidence as to exact correlation. Fossils are very scarce and samples quite irregular in quantity and spacing. Mechanical analysis, calcium carbonate determinations, and heavy mineral have all been considered, as well as the lithology and fossils.

Results Obtainable with the Cenco-Concave Grating Spectrograph. J. B. DERIEUX.

The concave grating spectrograph has a focal length of 106 centimeters, a dispersive power of about 16 angstroms per millimeter, and a resolving power of 15,000. With the fine slit, the lines are very sharp and the sodium double lines are separated about $\frac{1}{3}$ of a millimeter and therefore the wave lengths may be determined to one angstrom or less.

The spectrum plates are 5 centimeters wide and 25 centimeters long. Seven exposures of about 5 millimeters each may be made on one plate, or 14 exposures with 2 millimeters.

Plates of spectra were shown projected so that the audience could see the sharpness and separation of the lines.

Experimental Evidence for Functional Autonomy of Motives. DOROTHY RETHLINGSHAFER.

The present experimental evidence for Allport's use of the principle of functional autonomy of motives seems inadequate as supporting the systematic and

long-lasting activities of human adults. An examination of the studies cited by Allport, and related work, would indicate that the techniques used are suggestive of how an approach may be made to the problems inherent in human motives, but as yet permit no conclusions in regard to the principle of functional autonomy applied to these motives. Certain experimental methods used in these studies are reviewed.

Effects of Organization upon the Remembering of Meaningful Material. CLYDE MONTGOMERY and KARL ZENER.

Traditionally the memory of meaningful material has been approached indirectly by inferences primarily from results obtained through the recall of nonsense items. Recently more direct attempts have been made to investigate the memory of meaningful verbal material as a function of its specific content and organization rather than merely in terms of its degree of meaningfulness. The present experiment investigates in a fashion more controlled than hitherto the factor of essentiality, or relevance, to the structure of a story.

Ten items were differentially embedded in two stories in such a way that five were essential to the plot of one story but irrelevant to that of the second, and the reverse. The stories were given to 12 groups of sixth and seventh grade pupils totalling nearly three hundred students. Reproductions were obtained after three time intervals—15 minutes, 24 hours, and 7 days. Each group was given only one story and was asked for only one reproduction.

This procedure made possible comparison of the recall of objectively identical items when they were and were not essential to the structure of the story in which they occurred. At all recall intervals the items were remembered better when they were essential than non-essential, the average recall being respectively 45 and 26 per cent. The relative advantage of the essential items increased with the recall interval. The differential effect showed in the recall of the details as well as in the main points of the statements and was strongest in the less well recalled. Finally the appearance, in the reproduction, of supplementary items not present in the original story was markedly affected by the factor of essentiality. Ninety-five per cent of such additions in the recall were qualifications of or related to essential items.

Some Experimental Problems in Military Psychology. A. G. BAYROFF.

The experimental psychologist will discover many interesting problems in connection with aviation. Some of these are: What factors are involved in visual acuity which are not involved in perception of figure-ground relations? What is the effect of the nature of the object on the perception of its movement? Can people be trained to increase the ease of their perceptions of form, movement, etc.? What are the factors that determine the perception of visual stimuli of brief duration? Is it necessary to include mental hygiene in the training of a student pilot so that he can learn to control his automatic compensatory movements? Is it necessary to direct the student's attention to every detail of his movements? What factors are responsible for the disintegration of the

motor coordinations in flying? Many more problems suggest themselves, and the experimental psychologist can not only make a contribution to the carrying on of the war but will also contribute to the solution of many psychological problems.

A Study of the Cytology of Toxocara cati Brumpt. C. M. ALLEN.

The taxonomic history of the common cat ascaris, *Toxocara cati*, is long and confused. It is frequently impossible to tell from the literature whether work has been done on *T. cati* or the dog ascaris, *T. canis*. The present paper is an account of an investigation on both the male and the female and in both the mitotic and meiotic cycles of this common parasite. The classifications of Yorke and Maplestone and of Faust were used. All methods were routine except that Delafield's haematoxylin was substituted in the Haidenhain schedule to secure greater contrast between vitellus and chromatin material in mature eggs.

Near the upper end of the testis cells are found passing through a period of rapid multiplication. In the metaphase plate there are nine chromosomes one of which is considerably larger than any of the others, measuring approximately 1.2 microns in length. Near the middle of the testis cells which have gone through a resting stage can be found. Here the largest chromosome measures 2.5–2.7 microns in length. Near the lower end of the testis cells whose nuclei exhibit meiosis can be found. Synapsis takes place in the prophase preceding the heterotypic division. The final division into functional germ cells or spermatids usually consists of a lateral constriction of the nucleus giving two end product cells with the reduced number of univalent chromosomes. Half the functional spermatids contain four chromosomes and the other half contain five of which one is the large unpaired element.

After the last division resulting in the reduced number of chromosomes the sperm nuclei return to the resting condition, in which stage they remain until after the eggs of the female have been penetrated and the fertilization figures produced. By the time the sperms have been extruded from the ductus ejaculatoris the amount of cytoplasm has been greatly reduced. After transference to the vulva of the female the sperms assume rather irregular shapes and seem to crawl up the uterus to the seminal receptacle in an amoeboid fashion. Actual penetration of the egg occurs as it is entering the upper end of the uterus. From this time until actual fertilization occurs, the sperm appears in the egg as an elongated, arrow-head-shaped body with an elliptical central apparatus consisting mainly of a large number of peripherally arranged chondriosomes.

Near the upper end of the ovary cells in mitotic division can be found. The nuclei average 5.5 microns in diameter and contain nine chromosomes one of which is a great deal larger than any of the others and seems to be of a double nature. By the time the egg has entered the uterus considerable vitellus has been added and after the sperm penetrates the egg the egg wall becomes thickened and shell-like. Fully formed eggs attain a size of 60–65 microns with shell 2.5–3 microns thick. The nuclei of these cells represent the primary oöcytes

and their metaphases show eight chromosomes, four globular and four rod-shaped, in addition to the large element which at this stage appears to be clearly of a double nature.

Synapsis takes place in the prophase preceding the first maturation division. After synapsis two divisions occur which bring about the reduced number of chromosomes in the resulting oötids. A split in the paired homologs produces tetrad figures. Actual reduction between homologous chromosomes takes place in the first or heterotypic division; the second or homotypic division merely separates elements of dyads.

The spindle of the heterotypic division is set at an angle to the wall of the egg and one group of dyads remains near the end of the spindle, forming the secondary oöcyte while the other moves close to the edge of the egg to become the first polocyte. Both the secondary oöcyte and the first polocyte now pass through a homotypic division in which the elements of the dyads are separated. Both the heterotypic and homotypic divisions take place rather rapidly and no definite vesicular nucleus is formed between them.

All of the functional ootids possess five chromosomes, one of which is much larger than the rest and homologous with the large chromosome found in half the functional spermatids. This is the sex chromosome and sex determination is of the XX-XO type of male digamety.

The Respiratory Mechanism in Turtles. F. H. MCCUTCHEON.

The diamond-back terrapin (*Malaclemys centrata*) was studied in an attempt to clarify various conflicting explanations of turtle respiration. Recordings, measurements, and observations of the factors involved in breathing show the following:

a) The valvate musculature of the glottis is so regulated that the glottis is closed except during expiration and inspiration. The intra-pulmonary pressure is, therefore, independent of atmospheric pressure, and records show transient pressures as high as +18.0 mm. Hg. It usually varied between +2.0 and +4.0 mm. during intervals between breathing cycles (apnea).

b) The breathing cycle consists of expiration, inspiration, and compression, followed by a variable period of apnea. For one 654.0 gr. female specimen observed at rest with the temperature 29°C., a frequency of 3.7 cycles a minute was average. From one to four or more cycles in series occurred with two consecutive cycles most common. The period of apnea for 108 cycles varied from 22 sec. to 46 sec. with an average of 31 sec. Larger variations in apnea were frequently recorded, sometimes lasting from 5 to 7 minutes when the specimen is entirely undisturbed.

c) Normal, quiet breathing is accomplished by two sets of muscular diaphragms which act antagonistically. The expiratory set consists of the transverse abdominis and the diaphragmaticus which practically enclose the viscera. They compress the lungs when contracted. The inspiratory set includes the serratus magnus which extends across the anterior leg pocket in the shell and the oblique abdominis which is similarly located at the posterior flank.

Most of the resting displacement of air and inspiratory activity is the result of movement of the posterior diaphragm. Forced breathing (dyspnea) involves increased amplitude of the oblique abdominis, more active movement of the serratus magnus, and, under some conditions, movement of the pectoral girdle and neck. Also the period of apnea is reduced or may be eliminated.

d) Records of throat movements indicate that they are associated primarily with olfaction. The studies of intra-pulmonary pressure and determination of the fact that the glottis is normally closed during apnea preclude any frog-like breathing function of the throat movements. This has been a major source of disagreement in the explanations of turtle breathing.

Similar conclusions were reached for the box turtle, mud turtle, snapping turtle, and loggerhead turtle.

Anatomy and Taxonomy of Two Species of the New Genus Pregermarium (Turbellaria, Alloeocoela) from the Beaufort (N. C.) Biological Station. MARGARET A. STIREWALT, F. F. FERGUSON, and W. A. KEPNER.

Pregermarium is a genus, belonging to the Cylindrostominae, that is protogynous. The ovary lies anterior to the cephalic ganglia. *Pregermarium beaufortense* and *P. carolinense* differ in that the former lacks bands of black pigment in the dorso-anterior region of the pseudocoele; its walls of pharynx and penis are stouter and its vasa differentia more slender. A posterior group of unicellular glands in each species suggests the byssus gland of certain molluscs. The implantation of a mass of spermatozoa into the epidermis of one specimen of *P. beaufortensis* by another is recorded. Chromosome number for each species is: x = 4, 2x = 8.

Notes on the Turbellarian Fauna of Rochester (N. Y.). Anatomy of Macrostomum ontarioense n. sp. F. F. FERGUSON.

Notes on the ecology and varieties of Turbellaria of Monroe County, N. Y., with special reference to the anatomy of *Macrostomum ontarioensis* n. sp.

Forage Utilization by the White-Tailed Deer of the Appalachian Region. E. A. SCHILLING.

Ecological studies were initiated on the Pisgah National Game Preserve eleven years ago for the purpose of determining the relationship of the deer to the environment. More specifically the studies were designed to determine what plants were eaten by deer and the relative palatability of the various species eaten. The stomach contents of many deer taken throughout the different seasons were analyzed and the findings were correlated with data obtained by charting of line quadrats in the various vegetative types. The following major types in order of total acreages involved were studied and classified from the standpoint of deer food value: oak-chestnut, cove hardwoods, yellow pine-hardwoods, northern hardwoods, fields, spruce balsam, lake, and barren. One hundred eighty-seven species of plants were found to be important as deer food. Only 40 of these were eaten in winter and only 16 of these 40 were considered

as important winter deer foods. Four species comprised 80 per cent of winter deer food. Important timber trees browsed extensively by deer during the reproduction stage are yellow poplar, basswood, white oak, red oak, maple, white ash and magnolia. Abnormally high deer populations can seriously damage forest vegetation. The solution seems to lie in keeping the deer herd within the carrying capacity of the range.

Thirty Million Acres of Undiscovered Wildlife-Land in the U. S. A. VERNE E. DAVISON.

Thirty million acres of agricultural land, unsuited for crops, range or timber, await a new classification. A new term "wildlife-land," is needed to designate these small bits of land which are intermingled with cropland and pasture, but not used for either purpose. Lands useful for wildlife and nothing else include such areas as steeply sloping stream banks, rock outcrops, field and woodland borders, ill-drained bottom lands, thick brush and scrub, swamp and marshlands, ponds and lakes. If such areas are frankly recognized as best suited for the production of wildlife and not forced into treatments where labor and money will only be wasted by misuse, farmers, wildlife and the nation generally will be the gainers.

A Program for the Management of North Carolina's Inland Fisheries. WILLIS KING.

The attention of the conservation minded public has quickly shifted from wildlife to winning the war. In order to retain the foundation of our conservation structure, it is as important that our resources be properly conserved in time of war as it is in time of peace. The attention given North Carolina's varied aquatic resources has in many instances been inadequate to conserve the aquatic life and perpetuate good fishing. Protection is recognized as one of the most important features of the fisheries program, and at least a third of the available revenue goes for this purpose.

Research activities are principally surveys of the streams in the western part of the state to determine their capacity for carrying trout and other game fish. This program, although begun in 1941, has enabled a better use of the fish produced at the state hatcheries, and has shown the need for modifying stocking procedures and the improvement of fish habitats. Special problems which await study include a survey of the waters of the Coastal Plain. This will require the development of new techniques. Water is being impounded by both private and public agencies at a rate faster than the state is able to provide fish for stocking the new ponds and lakes.

Another third of the available fisheries funds are being spent on fish hatchery maintenance and operation. The state's trout hatcheries are considered fairly adequate, but the facilities for rearing warm-water fish are insufficient. The development of new water areas promises to be the best means of increasing the available public fishing areas. An additional source of revenue beyond that obtained from the sale of fishing licenses and permits must be obtained before

the present program can be expanded. Legislation will be needed on this point, as well as to enable control of pollution which is severe in many portions of the state.

Even though the war may delay the achievement of many objectives, the need for providing increased sport and recreation is certain to arise when conditions again become normal.

The Accomplishments of the North Carolina Cooperative Farm Game Program Since 1937. JAMES C. DARSIE.

The North Carolina Cooperative Farm Game Program was initiated in July 1937 with a field biologist stationed in each of six districts in the state. Since that time 1273 agreements have been entered into with cooperating landowners in 85 counties. One hundred and twenty-six agreements have been cancelled. While the program is designed to improve the status of the wildlife resources in general, it gives most attention to the game species. Based on two long-range principles of providing a satisfactory environment and regulating the kill so as to conserve sufficient breeding stock, the program is essentially one of educational demonstrations. From the standpoint of individual cooperators, the program frequently has not come up to expectations, but from the standpoint of getting people fundamentally interested in wildlife conservation, it has been fairly successful. A main problem involves keeping up the interest of the cooperators. Many farmers object to posting their land because of fear of offending neighbors. Hunting allowed on cooperating areas has been on the conservative side. Compliance with regulations concerning stray cats and free hunting dogs has been fairly satisfactory. Much hard work remains to be done but many people interested in game are becoming conservation minded through this program.

Research Dealing with North Carolina Fur-Bearing Animals. JOE C. RABB.

Studies are underway to determine the present status of important furbearers in North Carolina. Records of fur dealers have been analyzed in detail, whenever available. Considering the state as a whole, the muskrat makes up 36% of the total catch, raccoon 24%, opossum 23%, mink 8%, with grey fox, red fox, weasel, skunk, and wildcat making up the remainder. These percentages vary over the four natural districts studied; the Mountain, the Piedmont, the Northern Coastal Plains, the Southern Coastal Plains.

Under the present system of reporting sales of raw furs it is difficult to arrive at a figure representing the total catch, since furs may change hands as many as five times within the state, before reaching the manufacturer.

Research now underway is directed toward acquiring information on the best season for trapping, so as to insure primeness of the pelt and not to encroach on the animal's breeding season. Collection of ecological and life history data are part of the study. Also experiments aimed toward the improvement of marsh for muskrat habitat are being carried out.

Zoological Factors in Deer Research. SETH GORDON, JR.

The research involved in a state-wide study of white-tailed deer shows the close relation between pure and applied zoology. Pen-held animals are satisfactory for study of certain problems, particularly diseases, but may fail to bring out basic ecological and life history factors. Determination of sex ratios is necessary to learn whether the females are in a productive state, and if both sexes or one should be taken by hunters. To determine percentage of does with young, a modified Ascheim-Zondek test has been used with some success.

Using wild trapped animals and those killed by hunters, weights and measurements have been made on 700 deer in North Carolina. The difficulties encountered in handling wild deer have been partially removed by experiments employing injections of barbiturates. Hypnotics of this nature prevent shock to the animals, which frequently occurs in handling live deer.

Examination of wild deer indicates a higher degree of disease and parasitism than the public usually supposes exists. Diseases borne by the deer which are transmissible to man include anthrax, contagious abortion, tuberculosis, rabies, foot and mouth disease. Diseases and parasites normally associated with domestic animals but which attack deer include necrotic stomatitis, foot and mouth disease, cattle fever tick, rabies, encephalitis, tuberculosis, and various parasitic worms.

It will be necessary to solve several basic problems in nutrition before obtaining a complete picture of the ecology and natural history of the whitetail.

The Mourning Dove in North Carolina. MARK H. TAYLOR.

Four study areas ranging from 1550 acres to 8000 acres in size were established, one each in the Coastal Plain, Lower Piedmont, Western Piedmont, and Mountain Region for the purpose of determining nesting and breeding habits, migration habits, and densities and fluctuations of mourning doves in North Carolina. The study of the nesting and breeding habits received most attention during 1940, the year covered in this paper. Three hundred functional nests were studied on the four areas and April, May, June, July, and August were found to be the important nesting months. Field data revealed the peak of nesting activities in all except the Lower Piedmont study area to be in May, while the peak was reached in June on the area near Raleigh, North Carolina. Study should be carried through more nesting seasons. One hundred and fifty-one or 50.3 per cent of the 300 nesting attempts were unsuccessful, with higher losses during the early part of the nesting season. This may have been due in part at least to lack of foliage and poorly constructed type of nest built by doves.

Measurements of length and width of male dove gonads revealed a direct correlation of gonad size with nesting intensity.

PROCEEDINGS OF THE ELISHA MITCHELL SCIENTIFIC SOCIETY

OCTOBER 14, 1941, TO MAY 12, 1942

410TH MEETING, OCTOBER 14, 1941

F. K. CAMERON: *Recent Progress in Whole Cotton Investigation.*
W. L. ENGELS: *Ocracoke Island and its Vertebrate Fauna.*

411TH MEETING, NOVEMBER 11, 1941

R. B. LAWSON: *Structure and Clinical Use of Vitamin K.*
S. E. SMITH: *Sorption by Polymer Films.*

412TH MEETING, DECEMBER 9, 1941

D. F. MILAM: *A Nutritional Study of a North Carolina Community.*
E. P. COOPER: *The Nature of Cosmic Rays.*

413TH MEETING, JANUARY 13, 1942

STERLING BRACKETT: *Epidemiology of a Schistosome Dermatitis.*
H. B. GOTAAS: *The Time Factor in Chlorine and Chloramine Disinfection of Swimming Pools.*

414TH MEETING, FEBRUARY 10, 1942

E. S. ADDISON: *Problems of Naval Gunnery.*
NATHAN ROSEN: *What is Gravitation?*

415TH MEETING, MARCH 10, 1942

E. H. NEWCOMER: *The Use of Colchicine in the Production of Better Plants.*
D. E. COPELAND: *Cytology of the Triturus (Salamander) Pituitary.*

416TH MEETING, APRIL 14, 1942

J. L. COE: *Methods of Study in North Carolina Archaeology.*
W. A. RICE: *Huronian Sediments in Ontario.*

417TH MEETING, MAY 12, 1942

F. N. LOW: *Some General Features of the Cerebral Cortex.*
H. C. MASON: *Standardization of Streak and Fixed Virus.*

The following officers were elected for the year 1942–43:

President—M. J. Rosenau.
Vice-President—J. E. Adams.
Secretary-Treasurer—R. J. Wherry.

THE INTERPRETATION OF THE DEVELOPMENT OF THE HYPOPHYSIS (PITUITARY BODY)

By B. F. Kingsbury[1]

As, in the process of reasoning the premises determine the conclusion, so in biology and in science generally the assumptions serving consciously or unconsciously as a basic approach largely determine the conclusions reached. The development of an individual organism resolves itself into Growth and a change in the character of growth, or Differentiation. The nature of the processes which lie back of these and the factors, intrinsic or extrinsic, that determine them are the fundamental aspects of the problem which development presents. The concept of the body as an aggregation of distinct and distinctive organs frequently engenders an illogical approach. The greater the interest in any specific organ, the more marked is apt to be the shift in emphasis. Thus, the intense interest which centers in the distinctive metabolic effects which that unique structure the hypophysis exhibits, has been responsible for an undue emphasis upon the adult mammalian structure in the interpretation of its development. This has led to a reversion,—indeed, a perversion,—of the logical sequence in developmental interpretation. The well-known morphology of the hypophysis in a typical mammal with its four regions and cavity, the "residual lumen", is correlated with the usual view that Rathke's pouch as an up-pocketing of the primitive mouth cavity meets an out-pocketing of the neural tube and thus establishes the customary morphological relation of the adult[2]. That such a statement is incorrect has been several times emphasized (Cf. Gilbert, 1934; Kingsbury and Roemer, 1940). A cursory survey of the early development of the head suffices to reveal that Rathke's pouch as such is but the mechanical expression of a complex of developmental growth factors. These in turn are clearly responsible for the existence of the morphological parts typically recognized in the mammalian hypophysis. The recent recognition (e.g., Wislocki and Geiling, 1936) that a Pars Intermedia or a Pars Tuberalis is lacking in the adult of certain mammals is thus not as disturbing as might seem. Considering only the Pars Intermedia, it is clear that its presence as a subdivision of the hypophysis rests primarily upon (a) the presence of the residual lumen and (b) a close association with the Pars Nervosa. When these are lacking a pars intermedia as a morphological entity does not exist. A slight variant in the growth could produce the different morphology. The comment of Van Dyke (1939, p. 10) that: "The embryonic development of the organ in the

[1] The writer wishes to acknowledge the many courtesies extended by Dr. W. C. George and staff of the Department of Anatomy, including free use of all facilities of the laboratory and of the library.

[2] The most recent as well as most extreme illustration of this is found in a comment upon the presence at birth of a cranio-pharyngeal canal in a hybrid dog. "The posterior segment of the basisphenoid, which contains the sella turcica on its surface, shows a large opening which represents an arrest in the closure of the opening, Rathke's pouch, through which the hypophysis grows from the stomodaeum up into the cranium."

porpoise is therefore apparently different from that in other mammals," is thus hardly justified. The uniformity of the morphogenetic pattern in mammals does not suggest any fundamental difference in these particular instances. The primary transformation is that the superficial ectoderm immediately bordering the neural plate anteriorly, in the great expansion of the prechordal neural tube, becomes the hypophyseal area anterior to the pharyngeal membrane (oral plate). This ectoderm is primarily or secondarily in direct and intimate contact with the floor of the forebrain and tends to maintain this relation despite the intrusion and growth of mesoderm elsewhere. This is an essential feature of the morphogenesis as determining the presence of a Pars Intermedia. A more extensive early intrusion of mesoderm would determine its absence and establish the peculiar relation reported (among other vertebrates) in the whales and the porpoises. The embryology of these mammals is unknown, but the development of the hypophysis of the armadillo, another 'atypical' form, has been partially established (Oldham, 1941).

As has been indicated, Rathke's pouch, as such, is the mechanical expression of the great expansion and rotation of the forebrain, together with the intrusion and growth of the mesoderm. These, together with a certain grade of intrinsic growth, transform the ectoderm of the hypophyseal area into a pocket. The source and growth of the mesoderm (Cf. Adelmann, 1932) is such that an intrusion to separate the hypophyseal plate (or Rathke's pouch) from neural plate (forebrain vesicle) is in the typical mammal late, slight or often in part lacking. The presence of a cavity within the growth-folded ectoderm, remaining in the typical mammal as the "residual lumen," is also a mechanical expression of the mode of growth,—of negative character and of no intrinsic significance.

Both the vesicular character of the forebrain and the time of appearance and growth-expansion of the mesoderm are subject to marked variation within the vertebrate phylum. Hence the correlated morphology of the hypophysis is likewise subject to an equally extreme variation. In terms of early vesicular expansion of the forebrain, the different classes and orders of the vertebrates may be roughly arranged in two groups, as follows: Group I: Elasmobranchs (Selachians, Plagiostomes), Mammals, Reptiles, Birds, Myxinoids (Cyclostomes), Apoda (Caecilia) Crossopterygians (?). Group II: Crossopterygians (?), Lamprey (Cyclostomes), Dipnoans, Ganoids, Caudate and Anuran Amphibia, Teleosts. Only in the first four of group I is a typical Rathke's pouch found and only in the first two is the residual lumen usually persistent or typical. No Rathke's pouch exists in group II although in a number of classes a cavity comparable to the residual lumen may appear secondarily.

In elasmobranchs the characteristic and highly vesicular expansion of the brain, together with the correlated development of the hypophysis, is well known through the comprehensive works of Baumgartner (1915), de Beer (1926) and Woerdemann (1914). Norris (1941) has recently correlated knowledge of the elasmobranch hypophysis and has further given, with full illustration, an important and systematic presentation of the morphology of the structure in the entire class. Figures of earlier stages of the morphogenesis in the shark were

presented by the writer (Kingsbury, 1922, figs. 17–26). The hypophyseal plate is in contact with the floor of the forebrain from optic chiasma to infundibular recess. Its "folding off" by the growth of the mesoderm is peculiar in that it occurs at both its anterior and posterior ends, producing a capacious sack which is finally cut off from the superficial ectoderm. This 'double evagination' in the elasmobranchs led naturally to distinctive interpretations by the four investigators mentioned above.

Recognizing three characteristic lobes in the growth of the hypophyseal sack, —namely the anterior, inferior (ventral) and superior (intermediate) lobes, Baumgartner (1915) states that: "Rathke's pouch forms the posterior part of the anterior lobe. The later evagination of the ectoderm anterior to this forms the middle portion and the anterior extremity of the anterior lobe. . . .The inferior lobes develop from the lateral sides of the posterior extremity of the anterior lobe, i.e., from the Rathke's pouch. The superior lobe is from the caudal (superior) end of the hypophyseal anlage [i.e., from Rathke's pouch]". de Beer (1926) comments (p. 70):—"The maxillary processes in their development push the skin away from the brain and the folds so formed lap over Rathke's pocket (Fig. 88). Eventually when the folds have met beneath it and fused, the hypophyseal cavity is closed off, but it consists of more than the original invagination of Rathke's pocket. As in the pig and the lizard and presumably all Amniotes a portion of the outside world is nipped in in the closing of the hypophyseal cavity." Woerdemann (1914) recognized in elasmobranchs an original caudal evagination, Rathke's pouch. Later, anterior to this another evagination occurs, while an oral cavity space between these is included within the hypophyseal sack which thus is composed of an "anterior space", a "middle space," and Rathke's pouch. From Rathke's pouch he derives the superior lobes. The anterior lobes represent the anterior and middle spaces, while from the latter the ventral lobe(s) outpocket. Norris (1941) considers it sufficient to state (p. 38) that "The plagiostome hypophysis cerebri is a derivation of Rathke's pouch and the immediate environment of the latter." However, two pertinent figures (figs. 20, 21) are presented and in the description of the first of these he says: "This figure confirms the statement of Baumgartner and shows that the anterior part of the anterior lobe originates not from Rathke's pouch proper, but from an independent fold in the ectoderm anterior to and distinct from Rathke's pouch."

It is evident that the customary conception of the development of the hypophysis is hardly applicable in the elasmobranch where an apparently different mode of formation has been well established, determining an equally distinctive and complex morphology, not to be directly compared[3] with the relatively simple type of the typical mammal. The comparison has, of course, been attempted (Cf. Norris, p. 10). Woerdemann (1914) believed that the same tripartite hy-

[3] (Norris, 1941, p. 38.) "The plagiostome [elasmobranch] hypophysis has no exact detailed homologue in other vertebrates The lobes of the hypophysis, however varied in size, shape or situation, or in attachments and other relations to brain, saccus vasculosus and cranial walls, always conform to the definite plagiostome hypophysial type. The type is never obscured."

pophyseal origin which he found in the elasmobranch was also encountered in reptiles, birds, and mammals. However, the anterior space and the middle space are of progressively lesser relative extent in these three classes. The quotation from de Beer (1926) previously given similarly presents the same interpretation. There is of course a factual basis upon which the interpretation rests. The fallacy in logic lies in giving individuality and a name to a variant expression of a growth transformation and making this in turn a basis for homology. Thus, in all three of these classes alike, the hypophyseal ectoderm is in contact with the floor of the forebrain from chiasmatic ridge to the infundibular recess. The early expansion, rotation and bending of the brain, together with the growth of the mesoderm, effect the folding off as an hypophyseal vesicle or sack. If the mesoderm early appears anteriorly and expands[4] without markedly intruding between hypophyseal plate and the neural tube, an anterior pocket is established in addition to an earlier caudal pocket (i.e., Rathke's pouch). This has been shown to be particularly marked in the elasmobranch. It is clear in the lizard and snake among reptiles, but—in the opinion of the writer—questionable in birds. The anterior pocketing doubtless occurs in some mammals but it is certainly not obvious in others. There is no justification for giving the term 'Rathke's pouch' other than a descriptive connotation.

The forebrain in the animals of group I expands not only rostrally but bilaterally, the evagination of the optic vesicles early determining this aspect. It may be expected that the hypophyseal expansion shares in the bilaterality of growth and that variants in the degree or early expression will now and then be encountered. Such indeed is the case. Lateral expansion as the "lateral lobes" generally appears relatively late. However, it may occur early and take the form of separate and lateral 'evaginations',—as in certain lizards (Gaupp, 1893) and mammals (Chiarugi, 1894; Weber, 1898) and in the Apodan, Hypogeophrys (Laubmann, 1926). The last form will be referred to subsequently.

As was earlier stated, the cavity of the hypophyseal sack has no intrinsic significance; it is merely an expression of the mechanics of development. It is therefore not surprising that the residual lumen, as far as is known, becomes lost in birds (Rahn and Painter, 1941) and probably in most reptiles. As was earlier commented, this seems also true of certain mammals; at least it is not present in the adult. Even in the elasmobranchs, in which the hypophysis develops as an extensive sack, the residual lumen may become lost. Norris (p. 38) states that: "In the batoid type the entire hypophysis [pars buccalis] is solid, except for occasional vestigial persistencies of the residual cavity."

Finally, there are two unique vertebrate forms which have been included within group I, since the forebrain is formed and expands as a hollow vesicle and the hypophysis arises as a hollow sack. In the first of these, the myxinoid fishes, the hypophysis is an extensive sack resembling superficially that of the

[4] Haller (1924) and more specifically Haller and Mori (1925) who compare the hypophyseal development in elasmobranchs, reptiles, and mammals, consider that a variation in the anterior (rostral) growth of the Maxillary Processes determines the different morphology. The present writer prefers to homologize less sharply the mesoderm involved.

elasmobranch. However the cavity, from the beginning, is in free communication with the exterior in conjunction with the "monorhinic" nasal sack. As in the case of the elasmobranch, it is not to be readily and fully homologized with the hypophysis of the higher vertebrates. Furthermore, there is also established, apparently secondarily, a communication with the pharynx (foregut). The development is known only through some 12 stages (v. Kupffer, 1900, 1906) of one species (*Bdellostoma Stouti*) and further work is needed to clarify many points.

In the myxinoid just considered, the morphology and morphogenesis are unique; in the second form, the amphibian Hypogeophrys, the cephalic growth is typical, the cavity of the forebrain appears early and a Rathke's pouch is formed (Laubmann, 1926) though its cavity is slight and early disappears. In addition, as already noted, two lateral 'up-growths' become included together with a slight area anterior to the definitive Rathke pouch. Earlier stages are inadequately known. The crossopterygian fishes possibly belong in group I but their development is unknown.

In the remaining classes of the vertebrate phylum, group II, the epithelial constituent of the hypophysis is typically a solid body. Furthermore,—and again typically,—it developes as a solid mass or ectodermal wedge. The contrast in hypophyseal development as between the elasmobranch at the one extreme and the teleost at the other is so great that a fundamentally different morphogenesis might be suspected were it not for the obvious unity in structural pattern which the vertebrates present. There are however certain definite developmental differences which distinguish the two groups and which are of importance for an understanding of hypophyseal development. In the group just considered (group I) the eggs are relatively large and yolk-rick or (in mammals) follow the developmental pattern of a large yolk-rich egg. The central nervous system is formed by the free folding of a neural plate to a tube. In its growth in length the rostral neural plate (and later the vesicular forebrain) freely overgrows the surrounding territory with a marked head-bend. A characteristic hypophyseal plate is thus produced, the transformation of which to a hollow hypophyseal sack has just been reviewed. In group II the eggs are predominantly smaller through relatively yolk-rich; the transformation of the neural plate is massive and cavitation is retarded or late. The neural primordium "hugs" the substrate with the result that the cephalic over-growth is not free or only becomes free relatively late. A head-bend is late or less extreme. The oral cavity (so-called stomodeum) is accordingly late or masked. A further feature of significance is the precocious differentiation in the head ectoderm of a superficial and persistent keratinized layer set off from the deeper blastic or germinative layer. Whether this differentiation is correlated with early hatching and the beginning of a free-living life epoch need not be considered here. In any event, as compared with group I, it is linked with a modification in the mode of origin of certain ectodermal head structures,—lens of eye, otic vesicle, olfactory or nasal sack and the hypophysis, which thus does not originate as a folding of the entire ectoderm.

Necessarily, the hypophysis in its development shares the peculiarities of the general cephalogenesis, the mechanical factors determining its formation being correspondingly altered. In the degree of compactness and the correlated peculiar features of growth, the vertebrates included under group II present a certain gradation according to which they were arranged on page 147 and in this order they will be followed in a brief survey. It may be stated that in many instances additional details of the early hypophyseal development are desired. Although the developmental characteristics of Hypogeophrys places it in group I,—and the hypophysis was there described,—it is somewhat intermediate between the two groups; indeed it is the only form so far as known that may be so considered.

In both of the extant crossopterygians[5] a unique feature is present. In Polypterus (Waldschmidt, 1887; de Beer, 1926) and in the Calamoichthys (Bickford, 1895) a slight cavity is present in an otherwise solid hypophysis. The cavity is in free communication with the mouth cavity by means of a hollow stalk passing through a foramen in the parasphenoid bone. As the developmental stages are unknown it can only be surmised how it was formed. The inference is strong that there is here illustrated a persistent hypophyseal stalk.

In the lamprey (Cyclostomata) the early stages are quite well known (v. Kupffer, 1894; Woerdemann, 1914; de Beer, 1926; Tilney, 1937) but with some differences in interpretation. The early development is characteristic; a solid growth arises immediately rostral to the forebrain. It exhibits however a slight suggestion of a potential pouch formation and a hypophyseal cavity appears later but it is atypical. Furthermore, the adaptative growth of the upper lip underlying the characteristic monorhinia and the cyclostomic condition produces distinctive and unique relations (Cf. Kingsbury and Adelmann, 1924; de Beer, 1923, 1926). The hypophysis retains a connection with the ectoderm by means of a stalk which canalizes, putting the hypophyseal cavity in communication with the single nasal cavity. The lamprey, the myxinoid fish and the two crossopterygians just considered are the only known instances of a persistent communication with the exterior. It is possible, but not probable, that such a condition may be found in certain hybrid breeds of dogs (Stockard and Vicari, 1941).

In the dipnoans the hypophysis arises as a wedge from the ectoderm immediately anterior to the forebrain. In all three dipnoans a well-defined cavity subsequently appears within the solid mass (Dawson, 1940) occupying approximately the position of a residual lumen of a Rathke's pouch. This suggests a virtual cavity become real. Although it appears early (Kerr, 1933) it apparently never opens to the exterior; nor does the hypophysis maintain a persistent connection with the ectoderm.

The hypophysis of ganoids is more or less adequately known through studies of the development in Lepidosteus (Veit, 1924), Amia (Reighard and Mast, 1908; Smith, 1914; De Beer, 1923) and in Acipenser (v. Kupffer, 1893). It arises as a solid wedge immediately anterior to the rostral end of the forebrain

[5] Zoologists are not fully in accord as to the taxonomic position of these peculiar fishes. Some group them with the ganoids.

with which in Amia and Lepidosteus at least it is at first continuous. The connection with the point of origin is lost at or before hatching. A cavity appears quite early and persists.

In the remaining classes of the vertebrates (amphibia, teleosts) there is encountered no cavity formation of the character found in the forms just considered, where it suggests a potential primary cavity become real. Small cavities may appear, it is true (e.g., in teleosts), but they seem to be secondary cysts.

In the Caudata (Urodela) the solid hypophysis underlies the forebrain and is clearly derived from the ectoderm immediately anterior to the neural plate (Kingsley, 1905; Atwell, 1921). Published observations of the development have not included stages sufficiently early to give a record of relations to the anterior edge of the neural plate. However, observations by the writer in *Amblystoma punctatum* (unpublished) revealed an early continuity. In the anura the development of the hypophysis has been followed by several (Corning, 1899; Atwell, 1918; Schliefer, 1935; Kerr, 1939), although as in the tailed amphibia, the earliest development has been somewhat neglected. However, it is clear that it arises as a wedge of the blastic layer of the ectoderm immediately anterior to the neural plate (Corning, Schliefer), while in Bufo (toad) a primary connection with the rostral end of the neural plate is revealed (Schliefer).

In the teleosts, the peculiarities of the development of the head characteristic of the vertebrates of group II reach an extreme. This comment refers particularly to the early solidity of the forebrain and its close application to the substrate during the early stages of the growth of the head,—a period of overgrowth preceding the appearance of cavities in the brain and of the stomodeum. The obscure character of the developmental changes at this time, together with the technical difficulties, makes their determination difficult. This is clearly responsible for the fact that investigations dealing with the development of the teleostean hypophysis, from early classical papers (e.g., Hoffmann, 1884) up to the most recent (Woodman, 1939; Kerr, 1940), begin with stages in which the forebrain has become a vesicle and the typical relation to the hypophysis is already established. A solid cord or stalk may (at that stage) join the hypophysis to the stomodeal epithelium. It is not known whether or not the ectodermal material which becomes the (buccal) hypophysis arises anterior to the (solid) forebrain, as for example is the case in the bony ganoids, Amia and Lepidosteus. With these forms the teleosts might be expected to closely agree. A detailed examination of the early growth of the forebrain in its relation to adjacent ectoderm is needed to complete a comparison and establish a conformity with the other vertebrates of group II.

The apparently different mode of development of the hypophysis in the groups I and II has not escaped comment by those who have considered the matter broadly. There has been frequent recognition of the linkage of the type of hypophyseal development in group II with one or more of the characteristic features of head development earlier listed (Hoffmann, 1884, p. 92; Haller, 1924, p. 305; de Beer, 1926, p. 92; Tilney, 1937, p. 107). The comments are quite pertinent but the concept is customarily that of a gland arising from a definite

"anlage". The background is apt to be taxonomy and phylogeny and the mechanics of the growth is less considered.

As far as "phylogeny" is concerned, it seems clear that the hypophysis is a structure without a phylogeny under the customary use of the term. The "paleostoma" theory, in one form or another, is now of historic interest only and has not been seriously presented since 1923 (Neal); nor does the hypophysis represent a gland which has lost its duct. Morphologically it is mainly an expression of the mechanics of growth as determined by the developmental peculiarities of the prechordal region of the head. The differences in form and structure in groups I and II are largely linked with these. Groups I and II, it may incidentally be noted, do not coincide with taxonomic vertebrate divisions.

Aside from the differences which a varying developmental mechanics imposes, the developmental pattern is the same for all vertebrates, whether the development of the hypophysis takes the form of a Rathke's pouch 'folded off' as an extension of the stomodeal epithelium or a solid wedge appearing earlier with the growth changes less easily followed because of compactness. The two modes of hypophyseal development may now be compared, emphasizing the common features. Considering the hypophysis as an epithelial structure, the fundamental peculiarity is its contact with the floor of the forebrain to which the basal layer of the ectodermal epithelium is rather directly applied. Primarily the contact is at the anterior edge of the neural plate where,—in all forms adequately examined,—a continuity also occurs. Growth rapidly expands the forebrain and when the chiasmatic ridge and the primitive infundibulum become obvious, the hypophyseal epithelial contact is seen to be in this region. It is a customary interpretation that in such vertebrates as the fishes and amphibia the hypophysis "migrates" to the definitive position. This is without proof, and when it is recognized that all evidence indicates that the anterior region of the neural plate is primarily optical in its potencies (Cf. Woerdemann, 1929; Adelmann, 1936, p. 299), it is clear that there is very little shifting, the hypophysis but keeping pace with the growth expansion of the region and itself expanding. Experimental work is here needed.

In all vertebrates the appearance and growth of the mesoderm play a significant role in the morphogenesis. The degree of intrusion between hypophyseal epithelium and neural plate (tube) is characteristically different in different forms and frequently slight. At the caudal end of the contact the relation is, or becomes, most intimate; even a basement membrane, in the absence of intervening mesoderm, may be lacking and neural tube and hypophyseal ectoderm may be confluent, as Atwell (1916) early pointed out for the rabbit embryo. The significance of this intimate association has received scant attention, only Gilbert (1935) giving it serious consideration. It marks the region of the Pars Intermedia,—a term quite inappropriate if all vertebrates are considered, as Tilney (1937) pointed out. Even in this region mesoderm may intervene (e.g., birds and some mammals) and the region as such become non-existent.

Any detailed consideration of the cell types which appear in the development of the hypophysis would be, in the opinion of the writer, premature at the present

time. The gland concept of the hypophysis which views each cell type as a specific secretory source of a definite hormone or hormones cannot resolve the histogenetic aspect of the problem. Although supported by a great amount of work, cytologic and experimental, there are many lower vertebrates in which the cell types are inadequately known. Statements of different investigators are often inconsistent. The work of Scruggs (1939) reveals how variable the cytology (and morphology) may be within a single class (the teleosts). The three cell types so characteristic of the mammalian hypophysis,—acidophiles, basophiles and so-called chromophobes,—have been recognized in all of the classes of lower vertebrates, save perhaps the Cyclostomata. There are, however, pertinent differences. Cells which may be designated as chromophobes are often reported as rare or lacking; and the reason for this is quite apparent. Chromophobes may be described as "faintly basophile". Basophilia characterizes the undifferentiated cells of the early hypophysis and it may thus become difficult or impossible later clearly to distinguish two distinct basophilic cell types. The chromophobe of the mammal is now generally regarded as a "stem cell"; that is, one closest to the embryonic cell type. As a corollary to this it may be suggested that the basophile (at least as far as the basophilia is concerned) is an accentuation of the undifferentiated cell type. The acidophile alone would then express an obvious departure from the primary basophilic condition. The growth of the vasculogenic mesoderm is suggested as an important factor in its differentiation. Where in the mammal the region of intimate contact of hypophyseal ectoderm and neural tube remains, as in the pars intermedia, the basophilia is retained. In birds and certain mammals vascular mesoderm intervenes and the equivalent material develops eosinophiles. In the elasmobranchs and the teleosts the pars "intermedia" is vascular. In the elasmobranch (Norris, 1941) the cells next the vascular channels are eosinophiles,—a relation more than once noted in the mammal. In the teleost this relationship appears less characteristic; the cells bounding small cysts that may appear in development are eosinophiles (Kerr, 1940), while in the pars intermedia (so-called) the cells next the infundibular processes are eosinophiles (Hagen, 1936; Woodman, 1939).

It is obvious that additional detailed work is necessary to determine histogenetic correlations within the hypophysis. Doubtless the histogenesis of the hypophysis underlies the hormonal aspect of its metabolism, but it by no means follows that the cell types are "gland cells", and that the granules which are or may be present in the cytoplasm are specific secretory products. No one has expressed the advisable caution more tersely than Cowdry (1934, p. 195): "The supposition is not warranted that either the acidophile or basophile granules constitute secretion antecedents of hormones. . . . They may be by-products of some sort conditioned by the special chemical and physical changes in the two cell types, not the ripening active principles themselves".

However, it cannot be chance that the hypophysis,—or indeed any one of the so-called endocrine organs,—arises where and how it does. Its hormonal potentialities must have been determined in correlation with the developmental factors of its origin. The hypophysis originates from head ectoderm immediately

anterior to the neural plate. Whether its metabolic characteristics link in with a limitation in the differentiation of the nervous system: or with an alteration of the differentiation peculiar to the surface ectoderm cannot be said. The cell types[6] encountered in the epithelial hypophysis suggest the latter hypothesis. Possibly there has been a progressive advance in the endocrine potentialities of its cell metabolism. Nothing seems to be known regarding the possible hormonal potencies of the hypophysis in either the Cyclostomata or Elasmobranchii,—generally regarded as the most primitive of the living vertebrates. Danforth (1939, p. 336) makes the following petinent statement: "The complex endocrine system of higher vertebrates could hardly have sprung into existence in full functional efficiency. It must rather have had a significant evolutionary history, but one which, unfortunately, has thus far attracted scant attention." Further comments of that author reveal a full appreciation of the fact that the general problem of the endocrine organs and their hormones links in with the problem of metabolic unity.

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[6] No reference has here been made to the less frequent cell types often encountered in the hypophysis, such as squamous (keratinized) cells and ciliated cells bordering cavities. Acidophile cells and basophile cells of more than one type may occur. As was earlier stated, the significance of the cytological differences has yet to be analytically considered.

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A NEW FUNGUS ON CRAB EGGS

By John N. Couch

Plates 18 and 19

For two successive seasons Dr. C. L. Newcombe, Director of the Virginia Fisheries Laboratory, has sent me eggs of the blue crab (*Callinectes sapidus*) parasitized by fungi. The first season (fall, 1941) the eggs had been kept several weeks in sea water before they were sent and no indications of a parasitic fungus could be detected, except that on some of the disintegrated eggs the empty spherical zoosporangia of an organism resembling *Rhizophidium* were found. Again this past summer Dr. Newcombe and his associates noticed that some of the crab eggs failed to hatch and again, suspecting a parasitic fungus, material was sent to me for examination. This time a considerable number (2–5%) of the eggs were infected by an internal fungus which apparently belongs in the genus *Lagenidium*. Also a considerable number of the newly hatched living crabs had the mycelium in their bodies. With living material at hand it was possible to work out most of the details in the life history of the fungus and the stages as observed in the living but rather stale material were corroborated for the most part by material preserved in formalin immediately after removing the eggs from the crab. This fungus so far as I have been able to ascertain is the first member of the Lagenidiales which has been described from a marine habitat.

Lagenidium callinectes n. sp.

Mycelium developing entirely within the egg and eventually pretty well filling the egg, or body of the young crab, consisting of branched irregular hyphae which are sparingly septate, 5.4–12.6μ thick. Protoplasm with a pale whitish gleam and numerous oil bodies, becoming coarsely granular just before spore formation. In the process of spore formation and discharge the end of a thread becomes applied to the inner egg wall and swells to form a clavate structure up to 30μ thick. The part in contact with the wall forms a narrow tube which grows through the wall and immediately thickens and elongates to form a large tube 11–29 × 25–70μ. The tip of the tube gelatinizes and protoplasm flows out from the mycelium to collect into a spherical or subspherical undifferentiated mass surrounded by a thick gelatinous envelope. Protoplasmic mass up to 100μ thick. Spores formed as in *Pythium* from this mass of protoplasm, and becoming very active within the gelatinous layer before discharge. When mature breaking through the outer gelatinous membrane or vesicle and swimming sluggishly away. Vesicle persistent. Spores pointed at the front end and rounded behind with a diagonal groove which arises near the front end and extends backward over the rounded end. Cilia arising from this groove and one extending forward, the other backward while swimming. Zoospores about 9.6 × 12.6μ, with several oil globules one of which is distinctly larger than the others. Encysted spores oblong or subglobose, 10 × 11.3μ; monoplanetic. Resting bodies formed only after the crab eggs begin to disintegrate, apparently formed asexually in the threads, spherical, subspherical or oval, 18–30μ, thick, usually about 25μ thick, wall up to 3μ thick, containing pale whitish protoplasm and an eccentric mass of oil bodies; germination not observed.

Found growing parasitically on eggs and newly hatched young of Callinectes (common blue crab of eastern seaboard) collected by Mrs. Mildred Sandoz, July 7, 1942, East Lynnhaven, Virginia, and sent to me through the courtesy of Dr. C. L. Newcombe.

Infection is brought about by the zoospores which settle on the eggs (fig. 6). Unlike the spores of the Saprolegniales which round up when they encyst, the spore in this fungus encysts without rounding up. It germinates by sending a delicate tube through the outer egg covering (an extension of the stalk) and the egg membrane. Sometimes the latter is pushed in somewhat before its penetration is effected (fig. 7). Once the tube has entered the living part of the egg it enlarges to the full diameter of the hypha and as the hypha grows the cyst becomes entirely empty (figs. 7, 8). The empty cyst of the spore has a thick and persistent wall and can be recognized for several hours after infection. Several spores may infect one egg and it seems likely that the mycelium from one spore may produce several sporangia.

Because of the thickness of the eggs and their opacity, it has not been possible to follow the development of the fungus in the eggs. It is for these reasons that precise information on the rate of development of the fungus is lacking. However clusters of apparently healthy eggs placed on a slide in a drop of sea water showed several totally destroyed by the fungus after forty-eight hours. Such an egg is shown in figure 19. Even where there is no external sign of the fungus, heavily infected eggs can be distinguished from healthy ones by their smaller size and greater opacity (fig. 1). The healthy eggs were 281–298μ thick, while the diseased ones were about 231μ thick. A microscopic examination with brilliant illumination will show that in such infected eggs the hyphae are abundant, and the contents of the eggs have been pretty well destroyed. The mycelium is branched, sparingly septate and thin-walled. Indeed the walls are so delicate that if the egg is crushed or torn apart at this stage the hyphae are largely destroyed. The protoplasm in the hyphae has a pale whitish gleam with rather conspicuous fat globules.

The early stages in sporangial development have not been followed. Because of the septations in the mycelium it appears that one spore may give rise to a mycelium that forms several zoosporangia. As in other species of *Lagenidium* a zoosporangium may be formed directly from each segment in the mycelium. When a segment is to form a sporangium, the end of a thread grows against the egg wall to form a clavate structure from which a fine hypha is pushed through the egg wall (fig. 10). This hypha grows into a large cylindrical tube (fig. 11). When the protoplasm is ready to emerge the hyaline tip of the exit tube gelatinizes and enlarges to form a spherical mass of clear material into which the granular protoplasm flows (figs. 12–14). It takes 1–3 minutes for discharge to be completed. The mass of protoplasm is usually roughly spherical though sometimes it is quite irregular and never more than half fills the vesicle. From discharge of the protoplasm into the vesicle to dispersal of the spores occupies from 35–50 minutes. The protoplasm contains numerous small vacuoles and many minute fat bodies. Outlines of the spores become evident ten to fifteen minutes after discharge and a few minutes later the cilia can be seen growing out as short stubs (fig. 15). Twenty to twenty-five minutes after the undifferentiated mass of protoplasm has entered the vesicle, the spores have been formed and are swimming around individually in the vesicle (fig. 16).

This rather sluggish swimming may last for ten to thirty minutes before the vesicle bursts and the spores along with numerous granules are discharged (fig. 17). The vesicle is still evident for several hours after the spores have emerged.

The spores swim lazily with one cilium directed forward, the other backward. Dark field observations showed that both cilia are active in propelling the spore as in other biciliate members of the Phycomycetes (Couch, 1941). After swimming for a few minutes the spores settle, some on the crab eggs and others elsewhere. Only the ones on the eggs have been seen to germinate.

Several attempts were made to cultivate the fungus on agar. Zoospores were put on 0.5% plain agar, 2% plain agar, agar #5 (1000cc H_2O, 20 gms. agar, 3 gms. maltose, 1 gm. meat peptone), agar #F13 (500cc H_2O, 10 gms. agar, 0.75 gms. maltose, 0.02 gms. peptone) and corn meal agar but on all of these media the spores disintegrated instead of germinating.

The present fungus appears to be more closely related to *Lagenidium giganteum* than to any other known fungus. The coarse segmented irregular hyphae are very similar in both species. The two can be easily separated by differences in habitat, in the shape of the emergence papillae, as well as by the formation of a distinct vesicle and resting bodies in the present species and their absence in *L. giganteum*. These two species differ markedly from all other species of *Lagenidium* on the basis of size; both are much larger in all respects than other species.

As at present constituted the genus *Lagenidium* contains three rather distinct groups of organisms: (1) the single-celled species, as *L. oedogonii* Scherffel; (2) the rather delicate species with septate hyphae parasitic on algae, as *L. Rabenhorstii* Zopf or *L. Marchalianum* de Wildeman; and (3) the coarse species parasitic on animal substrata, as *L. giganteum*. Unfortunately not all of the species of *Lagenidium* appear to fit into such a scheme, e.g. *L. americanum* Atkinson. Sparrow (1939) in his discussion of a new unicellular species of *Lagenidium* parasitizing rotifer eggs points out the similarity of the unicellular species of this genus to *Lagena* but feels that more studies are needed before the genus *Lagenidium* is revised, a point of view with which the writer is in full accord. In such revisionary studies possible connections between the Lagenidiales and the Peronosporales should be kept in mind.

This appears to be the only member of the Lagenidiales so far described from a marine habitat. However, Atkins (1929) has described a related fungus growing in such a habitat as an internal parasite of the pea-crab (*Pinnotheres*). The *Pinnotheres* fungus apparently belongs in the family Saprolegniaceae, closest to the genus *Saprolegnia* or *Leptolegnia*, and may easily be separated from the present fungus on *Callinectes* by its non-septate mycelium and the *Saprolegnia*-like sporangia.

From this preliminary study it would be impossible to gauge the importance of such a fungus in the commercial raising of crabs. Careful studies should be made on distribution and extent of the disease, conditions that favor infection, host-parasite relationship, and possible means of control. For some of these studies it would be helpful to have the fungus in pure culture on agar or on other-

wise healthy crab eggs. Perhaps the fungus would grow on a nutrient agar made with salt water.

SUMMARY

A new species of fungus growing as a parasite on the eggs of the blue crab (*Callinectes sapidus*) is described as *Lagenidium callinectes*. In the material examined about 2–5% of the eggs and young crabs were infected. Infection takes place by the zoospores of the fungus which germinate on the surface of the crab egg sending in a germ tube which develops into a branched, sparingly septate mycelium. Each segment may become a zoosporangium. The undifferentiated protoplasm is discharged to the outside through a characteristically thick germ tube. The zoospores are differentiated within a distinct vesicle, are sluggish swimmers, monoplanetic, and apparently incapable of germinating except in contact with a crab egg. Spherical resting bodies are formed in old eggs. Attempts to grow the fungus in artificial culture failed.

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EXPLANATION OF FIGURES

PLATE 18

Fig. 1. Part of a cluster of eggs of *Callinectes*, showing central stalk to which eggs are attached, the two smallest ones parasitized by the fungus *Lagenidium*. × 100.

Fig. 2. One parasitized egg showing exit tubes of sporangium to right and empty cyst of infecting spore above. × 200.

Fig. 3. Cluster of zoospores in vesicle which is indistinctly visible. × 200.

Fig. 4. Parasitized egg showing two clusters of zoospores forming within vesicle; vesicle not visible. × 200.

Fig. 5. Resting bodies in egg, the membrane of which is visible above. × 200.

PLATE 19

(Figures were inked by Else R. Couch)

Fig. 6. Spores encysted on egg membrane. × 585.

Fig. 7. Spore cyst empty, germ tube elongating. × 1000. Fig. 8. Later stage of same.

Fig. 9. Spore settled not in contact with egg membrane, sprouted long tube which formed appressorium against egg membrane. × 585.

Fig. 10. Early stage in formation of exit tube. × 635.

Figs. 11–17. Stages in discharge of protoplasm into vesicle and formation and discharge of spores. ×290.

Fig. 18. Empty exit tube. × 635.

Fig. 19. Crab egg heavily infected with fungus. Note branched rarely septate mycelium; one sporangium at top about to discharge its contents; and four empty exit tubes. Semi-diagrammatic. × 290.

Figs. 20–22. Resting bodies formed after seven days. × 635.

PLATE 18

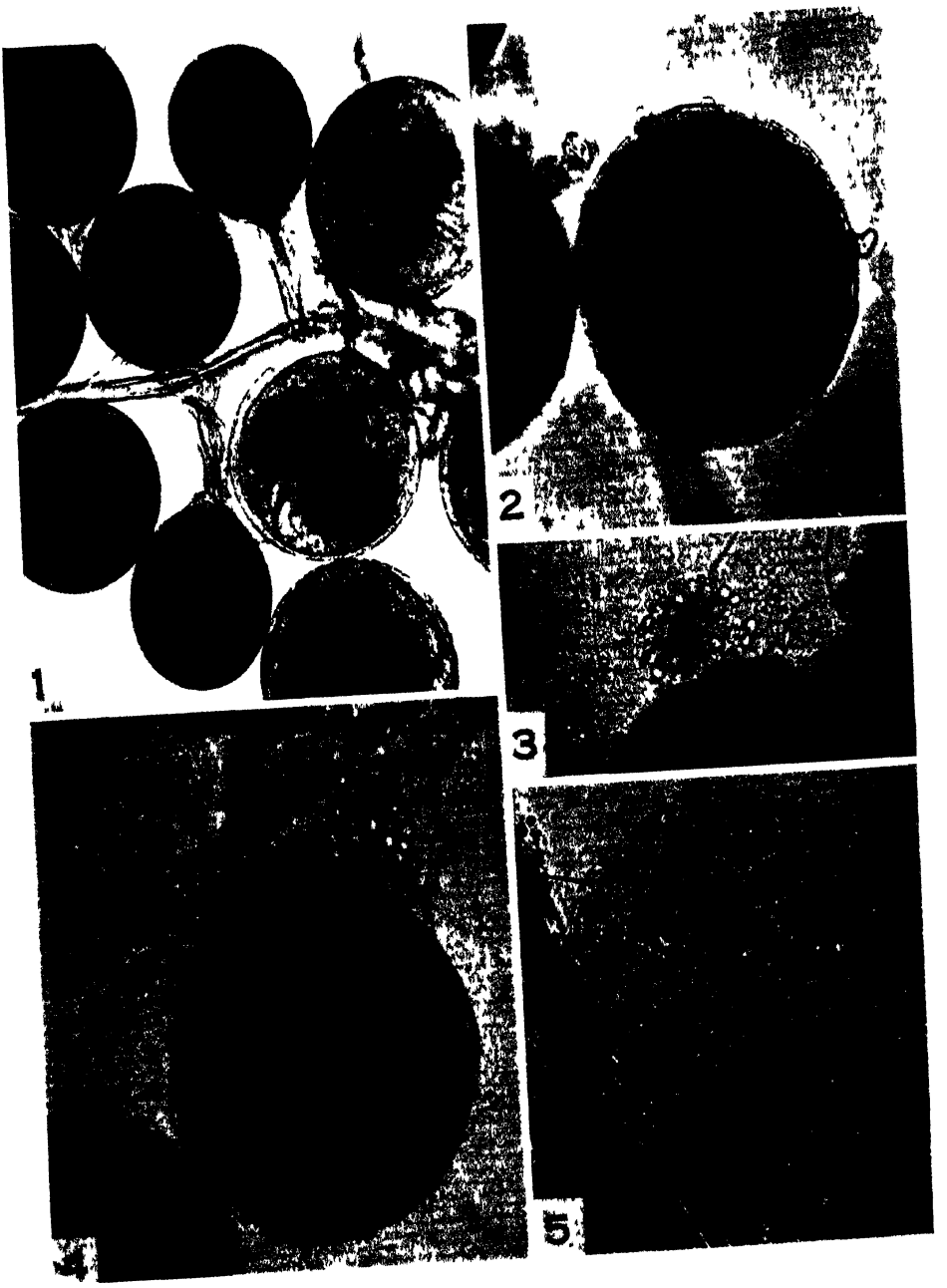
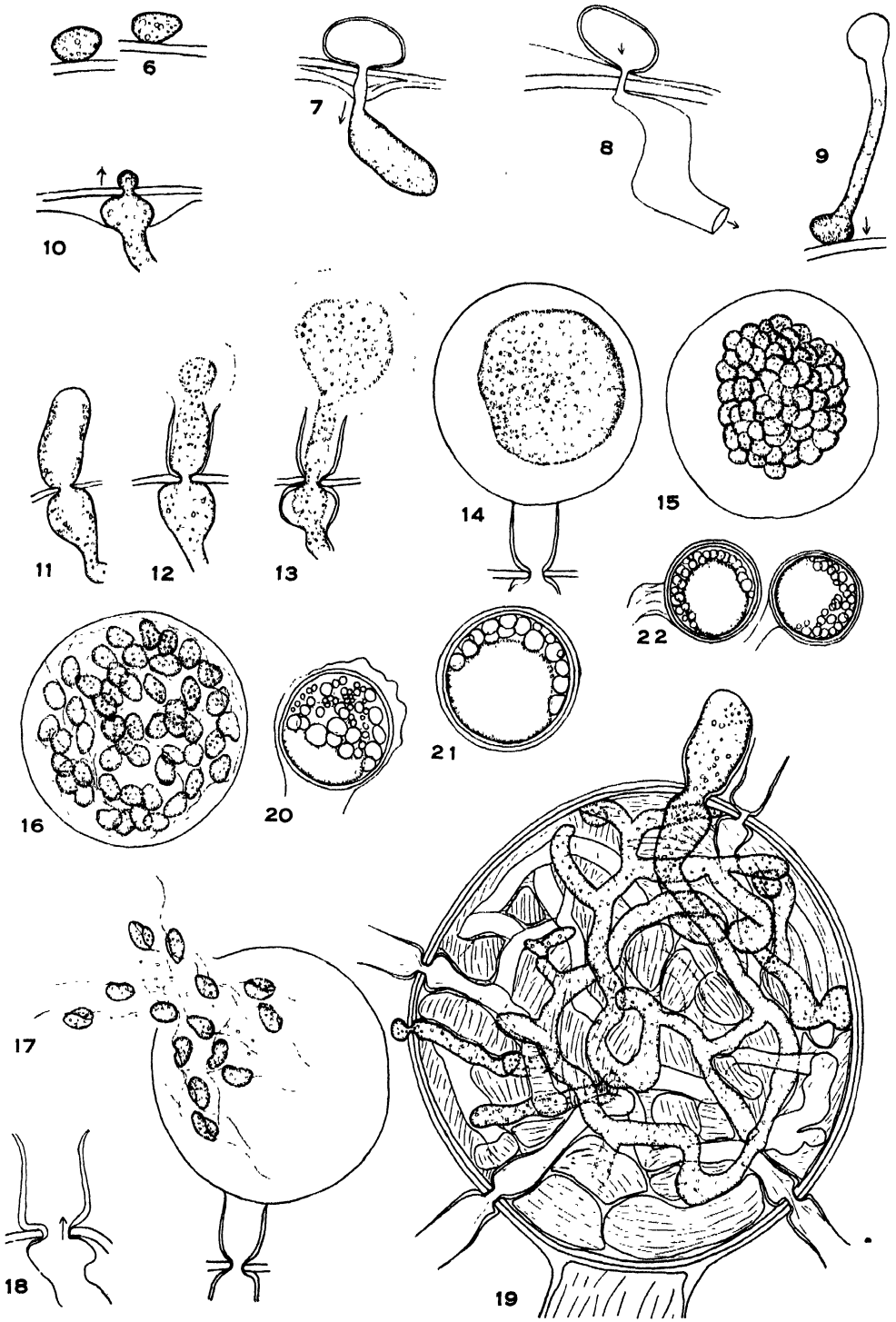


PLATE 19



FURTHER NOTES ON THE REACTIONS OF CERTAIN LOWER ORGANISMS TO THE COMMON SULFONAMIDES

By Edward M. Lavor and Frederick F. Ferguson

The following observations complete our study of the gross physiological reactions of certain free-living aquatic micro-organisms to aqueous solutions of the sulfonamides. We have felt that a better understanding of the reactions of the Protozoa and the lower Metazoa to these drugs might aid in the interpretation of their clinical toxicity. In general the toxicity of these drugs to the free-living forms studied followed very much the pattern which is expressed clinically. The lower forms exhibited a species variation in toxicity which was comparable to that shown in higher organisms. For example, the common laboratory ameba (*Ameba proteus*) displayed a marked resistance to all sulfonamides used. In a previous work Ferguson, Holmes and Lavor (1942, p. 53) reported the reactions of several micro-organisms to sulfanilamide, sulfathiazole, and sulfapyridine and included a few preliminary observations of the effect of sulfaguanidine upon these forms. These results had indicated that in general sulfaguanidine was far less toxic than the other three sulfonamides; that lower percentage solutions were lethal for *Hydra* sooner than the higher ones and that they affected the pigmentation of *Hydra*, readily producing a decolorization. This paper attempts to further the study of the unusual effects of sulfaguanidine upon a general fresh water micro-fauna and includes also a study of sulfathiazole as used upon *Mesostoma ehrenberghii* var. *wardi*, a well organized lower Metazoan (Turbellaria, Rhabdocoelida).

Series 1 of the sulfaguanidine tests consisted of a dilution range from 1.0% to 0.1% using distilled water as the diluent. Saturation was found to occur between 0.16% and 0.14%, thus many dilutions in the upper gradations contained crystals of the drug. Microscopic observations were made of each Stender dish with the contained solution and organism after 1, 2, 5, 12, 24 and 48 hours exposure. The higher (saturated) percentage solutions were not as toxic or lethal as the lower ones for the micro-organisms which in this case included *Hydra*, *Stenostomum*, *Halteria* and *Paramecium*.

Series 2 consisted of a dilution range from 0.50% to 0.05%. This range was used to ascertain the reactions of the organisms to a more dilute series of solutions. Again the lower percentage solutions were more toxic than the others for the microfauna. Within 4 days in series 1 and 2 a uniform toxicity was produced by all dilutions.

Since saturation occurred near 0.16%, this dilution was selected for use as a master solution from which the following twelve percentage solutions were developed: 0.16%, 0.144%, 0.128%, 0.112%, 0.096%, 0.080%, 0.064%, 0.048%, 0.032%, 0.016%, 0.0032%, and 0.0016%. Various series of sulfaguanidine dilutions were made up at these percentages using fresh distilled water, tap water (aerated) and aquarium water respectively, as diluents. In some cases the tops of the Stender dishes were removed during the test periods.

Series 3, using the dilutions as shown above, showed a relatively high toxicity throughout, undoubtedly due to the use of fresh distilled water as the diluent. Series 4, using a different distilled water, exhibited the same general reactions as observed for series 1 and 2. In series 5 employing aquarium water, the results were also the same as for series 1 and 2. The use of aquarium water as a diluent plus open dishes did not change the results as was indicated by series 6. Other series were used to determine the specific action of sulfaguanidine upon certain Protozoa and *Hydra*. In some of these last series there was developed an unusually high toxicity, while some showed a singularly delayed action of the drug. Summarizing the effects of sulfaguanidine in these last series, it may be said that by 24 hours' exposure very few *Paramecium* had survived, only a few rotifers were alive in the last three percentages, *Halteria* and various heterotrichs were normal, very few Crustacea endured throughout and the hardy ameba were dividing normally in all containers. *Stenostomum* did not seem to be affected by sulfaguanidine, but it was highly susceptible to the toxicity of other sulfonamides as shown in Ferguson, Lavor, and Holmes (1942, p. 54).

In general the action of sulfaguanidine on these micro-organisms was markedly erratic and opposite to that expected as compared with other observations upon sulfonamides. The entire lack of toxicity in certain of the micro-fauna when subjected to solutions of sulfaguanidine was indeed striking. *Hydra* may live for days literally surrounded by drug crystals, yet succumb in time in lower dilutions.

The effects of sulfathiazole solutions upon *Mesostoma ehrenberghii* var. *wardi* were next studied. This flatworm is a well developed, good-sized Turbellarian whose translucent body allows good microscopic observations. Control specimens were kept in distilled and aquarium water. Sexually mature specimens containing numerous eggs in various stages of development were subjected to a finely graded series of dilutions (range from 0.06% to 0.006%) and examinations were made after 15 minutes, 1, 4, 10, 24, 30 and 60 hour periods of exposure. All specimens were normal up to the 48 hour period, but within 60 hours a marked toxicity was apparent in the mid-range dilutions. In these middle lethal solutions the histological disintegration was complete and rapid. The following reactions were noted: the worms did not form new eggs during exposure, no eggs were laid during exposure, the egg cases became shrivelled suggesting an exosmosis, the animal's anterior end became swollen in some instances and in most there was a definite increase in a brownish parenchymal pigmentation. Because of the small number and extreme large size of the chromosomes in this form it would be good material in which to study cytological changes induced by sulfonamide drugs.

SUMMARY

1. Sulfaguanidine solutions were less toxic to fresh water micro-fauna than sulfanilamide, sulfathiazole or sulfapyridine.

2. Sulfaguanidine was not as uniform in its toxic manifestation as were the other sulfonamides studied. High percentages of sulfaguanidine appeared less toxic than lower ones.

3. The length of time required for the sulfaguanidine solutions to produce toxic effects did not appear constant.

4. Aquarium water, when used as a diluent, produced the same results as with distilled water, except that the effects were markedly deferred.

5. Sulfaguanidine solutions, when applied to *Hydra* were either non-toxic or only slightly so in higher percentage solutions; whereas they were weakly toxic in lower percentage solutions producing a uniform decolorization of tissues which did not appear to harm the animal.

6. *Mesostoma* when exposed to aqueous solutions of sulfathiazole exhibited the expected toxic reactions plus an unusual increase in parenchymal pigmentation.

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A STUDY OF THE GROSS PHYSIOLOGICAL REACTIONS OF LOWER ORGANISMS TO THE ANTI-SULFONAMIDE, PARA-AMINO-BENZOIC ACID

By Edward M. Lavor and Frederick F. Ferguson

The nature of the action of para-amino-benzoic acid (PAB) has been the subject of much investigation since PAB was reported to nullify the inhibitory effect of sulfonamides on bacteria in suspension (9). The closely related sodium para-nitrobenzoate when used upon *Streptococcus* cultures produces an inhibitory substance (7). PAB along with sulfanilamide and urethane possesses narcotic properties (3), seems to be an essential metabolite for autotrophic organisms (8), inactivates the fungistatic action of sulfanilamide (1), when combined with sulfaguanidine induces extensive thyroidal changes in the rat (6) and when used, among other subsances, upon *Cypridina* the luminescence is reduced (4).

In previous papers (2, 5), we have reported observations on the gross physiological reactions resulting in certain free-living aquatic micro-organisms when exposed to aqueous solutions of the sulfonamides. Since sulfanilamide (SA) responds readily to the inhibitory properties of PAB in clinical practice, this study was outlined to determine if PAB would inhibit these toxic manifestations of SA in the same organisms.

Many series of percentage solutions of SA and PAB were prepared, using freshly distilled water as the diluent. All of the test organisms were exposed to samples of the various dilutions of these drugs in small, clean, covered Stender dishes. Observations were made at slightly below room temperature. Living material included freshly collected freshwater ciliates, nematodes, and rotifers and laboratory cultured *Ameba proteus* and *Paramecium caudatum*. Microscopic observations were made with B. and L. wide field binoculars. Adequate controls were studied with each series of dilutions.

Series 1 contained 1 cc. each of SA and PAB of a dilution range of from 0.8% to 0.1%. This range was extremely toxic to all organisms with the exception of amebae within 15 minutes. Controls consisted of three dishes each of PAB and SA in the same dilution range. Here PAB proved to be generally more toxic than SA within this period. PAB of 0.1% is definitely far more toxic than SA of 0.8%. These results gave rise to the following questions: first, would PAB at a non-toxic or lethal concentration inactivate SA of 0.8%; second, would the non-lethal PAB concentration inactivate SA of a slightly toxic concentration; third, what is the lethal range of PAB on these forms?

Series 2 consisted of PAB (dilution range 0.1% to 0.01%) without SA. Within 15 minutes all organisms in the 0.09%, 0.08%, and 0.07% were dead; in the 0.06% only the hardy nematodes were still motile and in the other dilutions (0.05%, 0.04%, 0.03%, 0.02% and 0.01%) only amebae were assumed to be alive. Animals in the control dishes seemed unharmed, thus PAB in concentrations of 0.02% was definitely toxic, while in concentrations above 0.05% it was lethal.

Series 3 also consisted of PAB used alone but in a more diluted range (0.009% to 0.001%). Controls in this case were three dishes each of PAB (0.01%) and of distilled water. When observed after 15 minutes of exposure, the organisms in all dilutions seemed to be normal. In the controls the forms in distilled water were unharmed, while those in PAB (0.01%) were all dead except amebae. In our previous work on sulfonamides the amebae also displayed an exceptional resistance to the drugs. After 30 minutes the 0.009% dilution contained normal amebae but very few living ciliates and rotifers; the 0.008% had normal amebae, more living ciliates than above plus several rotifers and nematodes; the next two dilutions (0.007% and 0.006%) contained relatively few ciliates but unharmed amebae, while the remaining dilutions did not during this time lapse seem to be toxic. This series which seemed to be a suitable one was studied at the end of 1, 6, 24, 48 hours and then once daily for a week. It appears from our observations that the line between toxicity and non-toxicity lies between 0.009% and 0.008%. At the end of the study period the PAB control (0.01%) contained only normal amebae, while the distilled water contained normally dividing organisms. In the series only the 0.009% still showed its toxic effects on the ciliates in particular, the other organisms being normal. In all other dishes the amebae were increasing along with the remaining microfauna. It seemed desirable at this point: first, to select one easily grown form which exhibited a medium resistance to both PAB and SA; second, to expose this form (*Paramecium caudatum*) to a carefully graded series of SA dilutions, to ascertain its reaction not only to one such series but to several; and, finally, having found the near toxic dilution of PAB (0.008%) to combine this with a near lethal dilution of SA

Accordingly, series 4 was set up to consist of SA used alone on *Paramecium* with a dilution range of from 0.8% to 0.08%. Within 15 minutes this proved lethal for about 50% of the specimens in the 0.8%, the survivors in that dilution displaying slowness of movement and marked edema. Very few animals were dead in the 0.7% and 0.6% dishes, while in the other dilutions the majority were normal. After 1 hour of exposure the survivors in the upper part of the series resumed normal movement while the picture remained unchanged for lower dilutions. Thus, many immobilized organisms recover to the degree that they may be tactually stimulated to movement, however the abnormally swollen forms never regain normal activity.

Series 5 involved dilutions of SA which ranged from 0.072% to 0.008%. After 15 minutes of exposure the 0.072% dish contained *Paramecium* of which a few were swollen, some few exhibited pellicle papillae especially posteriorly while nearly all seemed partially paralyzed but moved if stimulated. This condition existed throughout the graded series down to the 0.032%, which dish had normal organisms excepting a few which still displayed tendencies toward immobility. In the next to last dilution few were slow and swollen, all others seemed normal. The last dilution (0.008%) contained apparently normal individuals. Within an hour's time there was a marked improvement for specimens in all dishes with only a slight abnormal edema showing in a few in the 0.072% dilution. Thus, in this series those individuals of *Paramecium* surviving the first few minutes have

a good chance of recovery from generalized cell edema, a sort of paralysis, vesicular swellings, or small surface papillae. Survivors gradually assume normality. It appears that in a medium range series the toxic effects of SA upon *Paramecium* may definitely be worn off in time.

Series 4 and 5 of SA were next combined as one (dilution range from 0.08% to 0.008%), adding 0.2 cc. of PAB (0.008%). This presumably should tell whether or not the extreme toxic effects in the upper range dilutions could be counteracted; on the other hand, the PAB plus the SA might show a toxicity in the lower range dilutions. Results after 15 minutes indicated that specimens of *Paramecium* in dishes from 0.8% to 0.056% were apparently unaffected by this combination, in the sense that PAB acts as an anti-sulfonamide, but the lower dilutions produced a marked slowness of movement and general debility. A repeat on the above extended dilution range showed again that the line between toxicity and lethal effects existed approximately at 0.056%. Our results did not show the expected inactivation of SA by PAB. Controls, consisting of PAB (0.008%) and of distilled water and combinations of equal volumes of these, showed that the combination was more toxic than either separately. PAB solutions in the range from 0.09% to 0.01% were either highly toxic or lethal. During a week's exposure to the combination of SA and PAB all organisms in all dilutions finally developed marked malformities with the cessation of divisions.

Since PAB does not nullify the action of SA under our described conditions it appeared that the next most significant thing to study was the nature of the toxic effects of PAB used alone upon *Paramecium* in a carefully graded series and to ascertain those concentrations of the acid which inhibit normal cell divisions. Our method was to place a definite number of regularly dividing specimens under exposure and to observe carefully the mortality and physiological effects including growth. Accordingly, series 6 consisted of 27 dilutions ranging from 0.1% to 0.001% of 2 cc. of PAB plus 0.2 cc. of *Paramecium* in culture water. In no case did the number of animals exceed 15. This extended series was repeated three times. Controls used were 9 dishes of distilled water plus a definite number of ciliates. These series were checked at 15 minutes, 30 minutes, 1 hour and then once daily for a week Results: during the first few minutes the line between toxic and lethal effects was at 0.008%, this level lowering to 0.003% at the end of an hour; in higher dilutions death comes suddenly leaving the cell in a well fixed condition; in the middle range the most noticeable physiological effect was slowness of movement; in several of the lower range dilutions many forms showed a peculiar edematous condition of the posterior cell producing there a small crooked tail. The distilled water of the controls seemed to be more toxic than usual, which might account for the somewhat increased levels of toxicity in the entire series. There was no increase in organism numbers except in a few dishes of the lower range. Within two weeks' time there developed in all dishes above 0.004% a delicate white fungus,* the appearance of which coincided with the disappearance of all microfauna.

* A description of this fungus is available for anyone interested.

SUMMARY

1. Para-amino-benzoic acid and sulfanilamide used alone and in combination in strong solutions (0.8% to 0.1%) are extremely toxic to free-living aquatic microfauna. PAB of 0.1% is definitely far more toxic than SA of 0.8%.

2. A dilution range of 0.1% to 0.01% of PAB is either toxic or lethal for most organisms studied.

3. Amebae are as highly resistant to the toxic effects of PAB as to the common sulfonamides.

4. A dilution range of 0.009% to 0.001% of PAB produces suitable gradations in physiological reactions including death, toxicity, and non-toxic effects. The line between toxicity and non-toxicity is on the average between 0.009% and 0.008% for most organisms.

5. *Paramecium caudatum* exhibits a medium resistance to both PAB and SA solutions.

6. *Paramecium* displays pellicle papillae, partial debility and decreased speed when exposed to a low dilution range of SA (0.072% to 0.008%). Those that survive the first few minutes in such dilutions eventually appear to recover. Organisms also recover from the toxic effects of PAB solutions.

7. Long exposure to a combination of PAB and SA produces cytological malformities and cessation of divisions in *Paramecium*.

8. In higher concentrations of PAB containing only *Paramecium* death comes suddenly as in fixation, while in low concentrations only a very few dilutions showed an increase in numbers of organisms. A fungus growth is in time supported by the higher concentrations.

9. Our study does not show convincingly that PAB under these conditions acts as an anti-sulfonamide, while its value as a growth factor is doubtful.

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